Cellular homeostasis

Proliferation ↔ Arrest

Survival ↔ Apoptosis

Undifferentiated ↔ Differentiated

Cellular homeostasis

Proliferation ↔ Arrest

Survival ↔ Apoptosis

Undifferentiated ↔ Differentiated

Differentiation. Morphogenesis.

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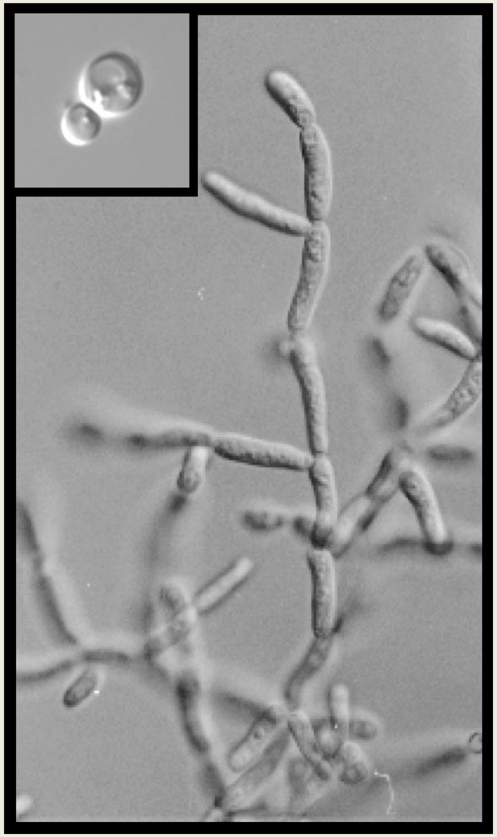
Dwight Kuhn ©

Nutrient-Dependent Foraging Behaviors

Filamentous Growth

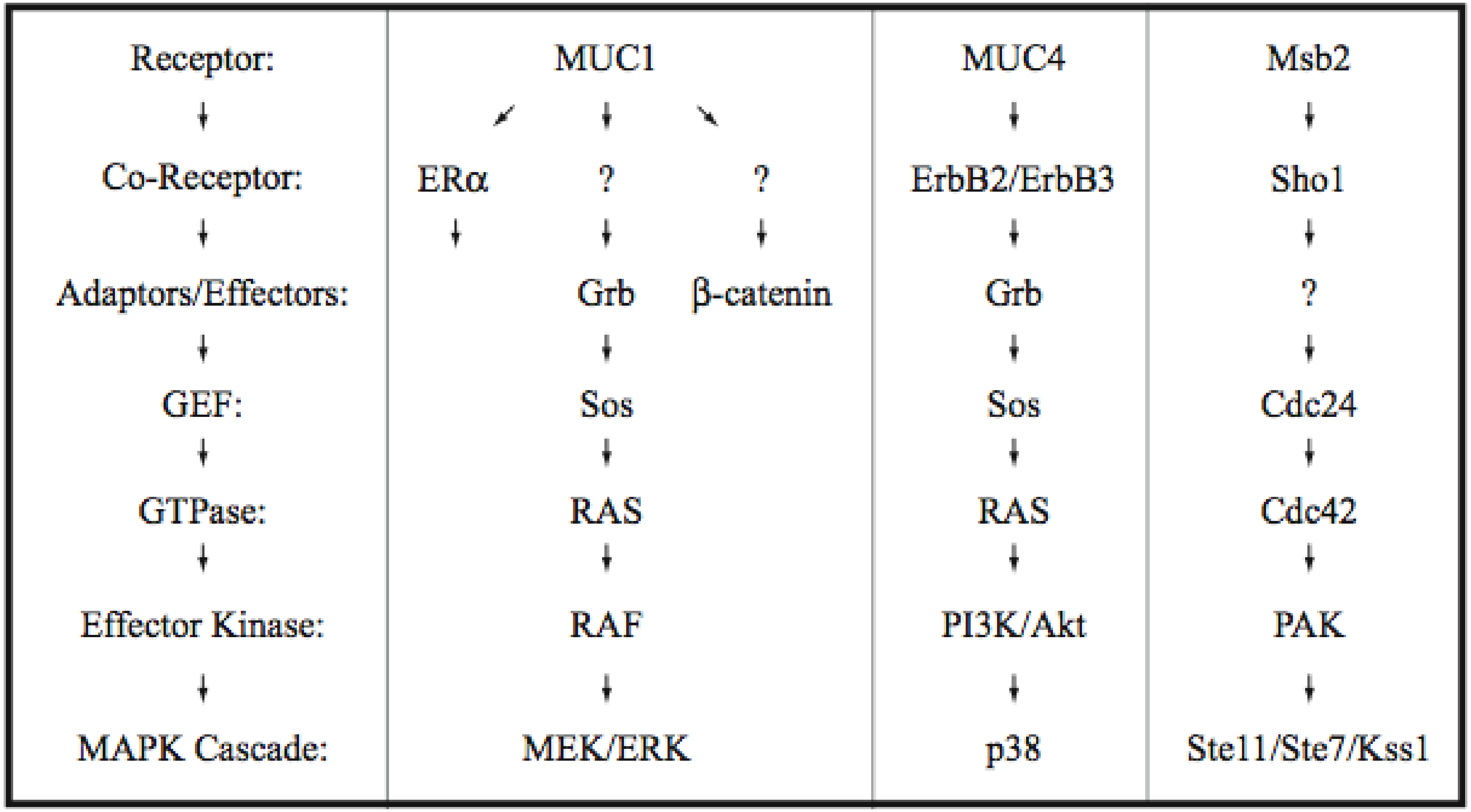


Biofilm Formation

T.B. Reynolds and G.R. Fink (2001)
V291 p878

Required for Virulence In Fungal Pathogens

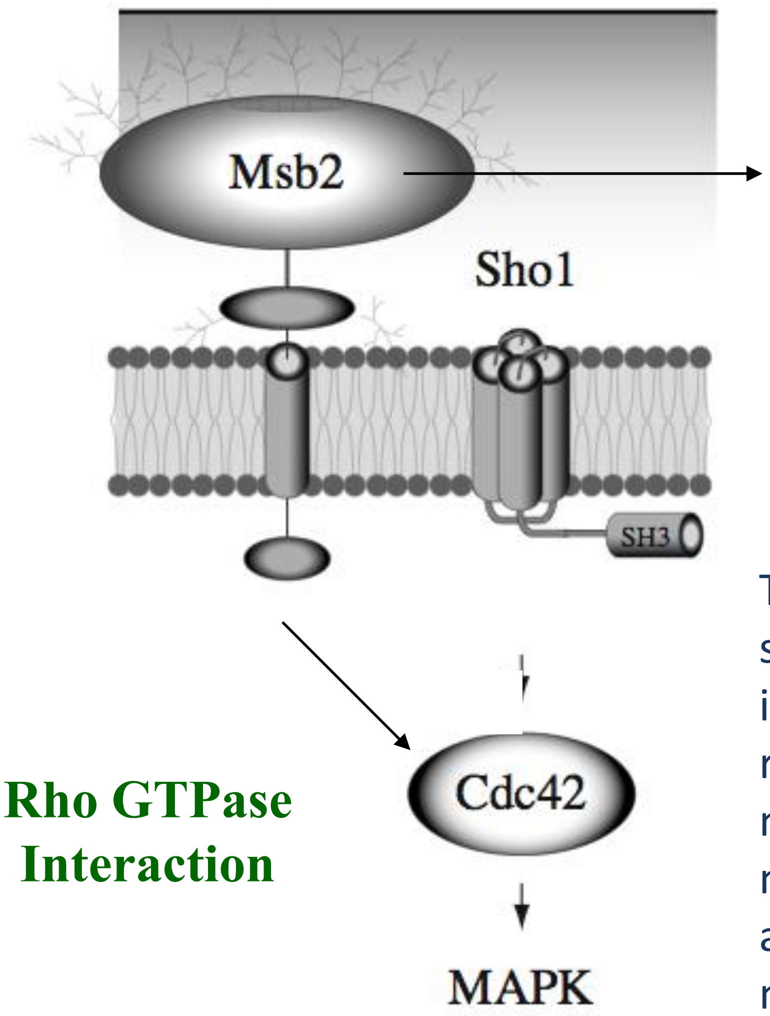
Studying Signaling Mucins in a Genetically Tractable Model May be Informative



Receptor:		MUC1		MUC4	Msb2
↓	↙	↓	↘	↓	↓
Co-Receptor:	ERα	?	?	ErbB2/ErbB3	Sho1
↓	↓	↓	↓	↓	↓
Adaptors/Effectors:		Grb	β-catenin	Grb	?
↓		↓		↓	↓
GEF:		Sos		Sos	Cdc24
↓		↓		↓	↓
GTPase:		RAS		RAS	Cdc42
↓		↓		↓	↓
Effector Kinase:		RAF		PI3K/Akt	PAK
↓		↓		↓	↓
MAPK Cascade:		MEK/ERK		p38	Ste11/Ste7/Kss1

Cullen, P.J. (2007)

Msb2 is a Signaling Mucin that Functions in the FG Pathway



Glycosylated

Transmembrane Domain

Rho GTPase Interaction

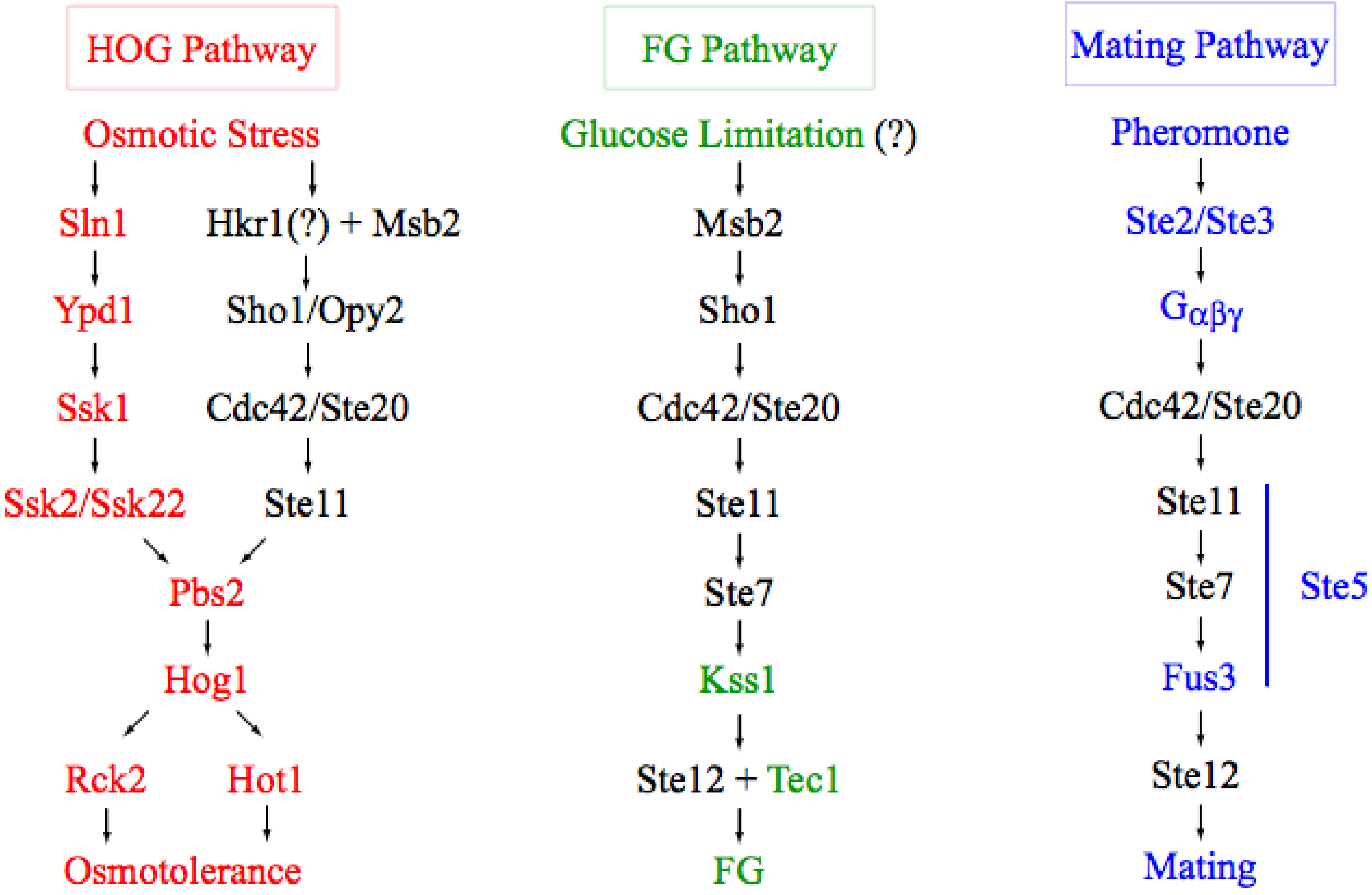
Tandem Repeats

N…(698)SQVSDTS
VSYTTSSSSVSQVSDTP
VSYTTSSSSVSQVSDTP
VSYTTSSSSVSQVSDTP
VSYTTSSSSVSQVSDTP
VSYTTSSSSVSQVSDTS
VPSTSSSSSVSQVSDTP
VPSTSSSSSVSQ(818)…C

The extracellular domains of signaling mucins may be inhibitory. Processing may represent a general activation mechanism for signaling mucins. Secreted/cell-associated aspartyl proteases may similarly process mammalian mucins (Liaudet-Coopman *et al.* 2006).

Cullen, P.J. *et al.* ***G&D*** (2004)

Signaling Mucins and MAPK Specificity



HOG Pathway

Osmotic Stress

Sln1 → Ypd1 → Ssk1 → Ssk2/Ssk22

Hkr1(?) + Msb2 → Sho1/Opy2 → Cdc42/Ste20 → Ste11

Pbs2 → Hog1

Rck2 → Osmotolerance

Hot1 → Osmotolerance

FG Pathway

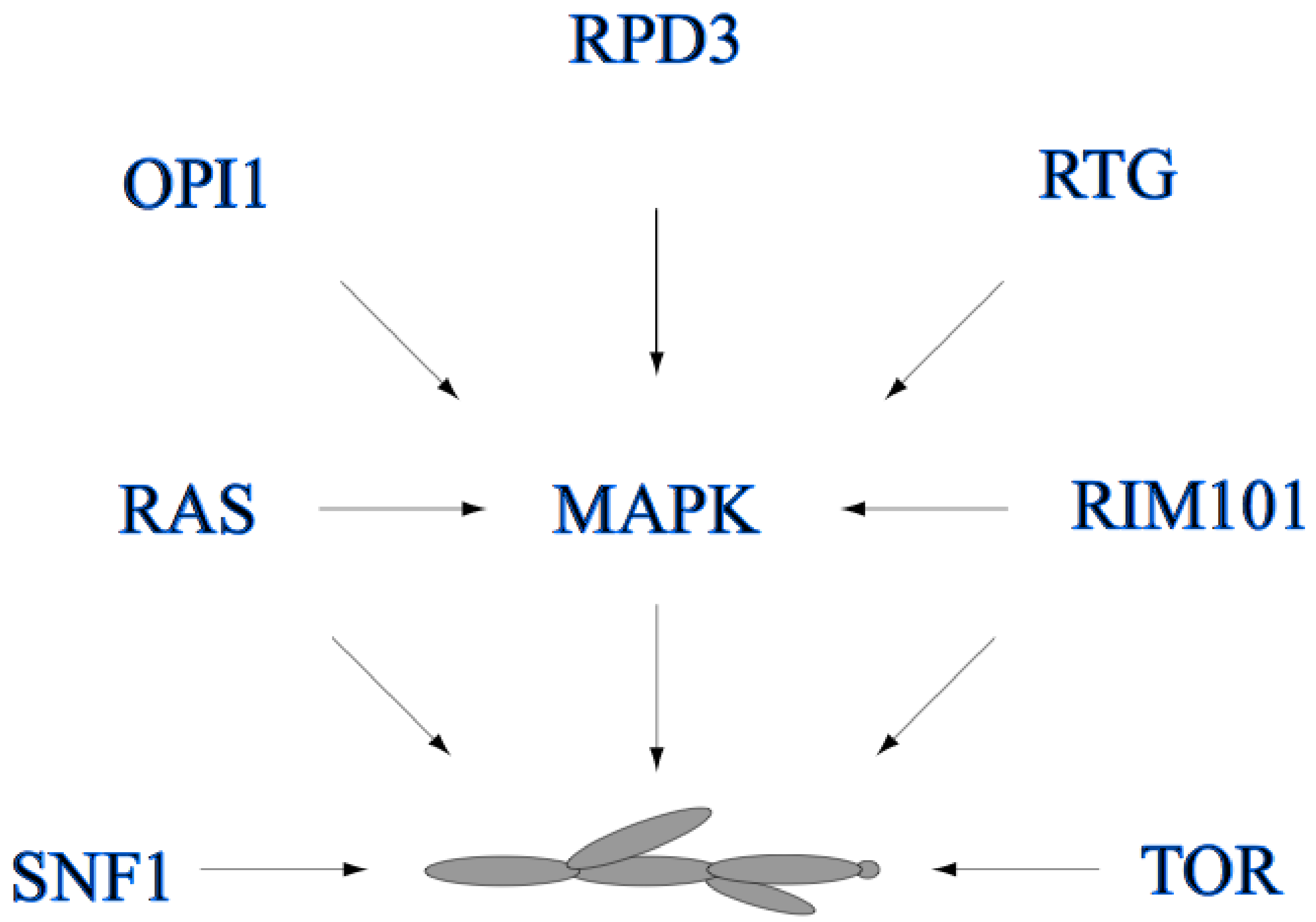
Glucose Limitation (?) → Msb2 → Sho1 → Cdc42/Ste20 → Ste11 → Ste7 → Kss1 → Ste12 + Tec1 → FG

Mating Pathway

Pheromone → Ste2/Ste3 → $G_{\alpha\beta\gamma}$ → Cdc42/Ste20 → Ste11 → Ste7 → Fus3 → Ste12 → Mating

Ste5

An Integrated Network of Filamentous Growth Regulation



RPD3

OPI1

RTG

RAS

MAPK

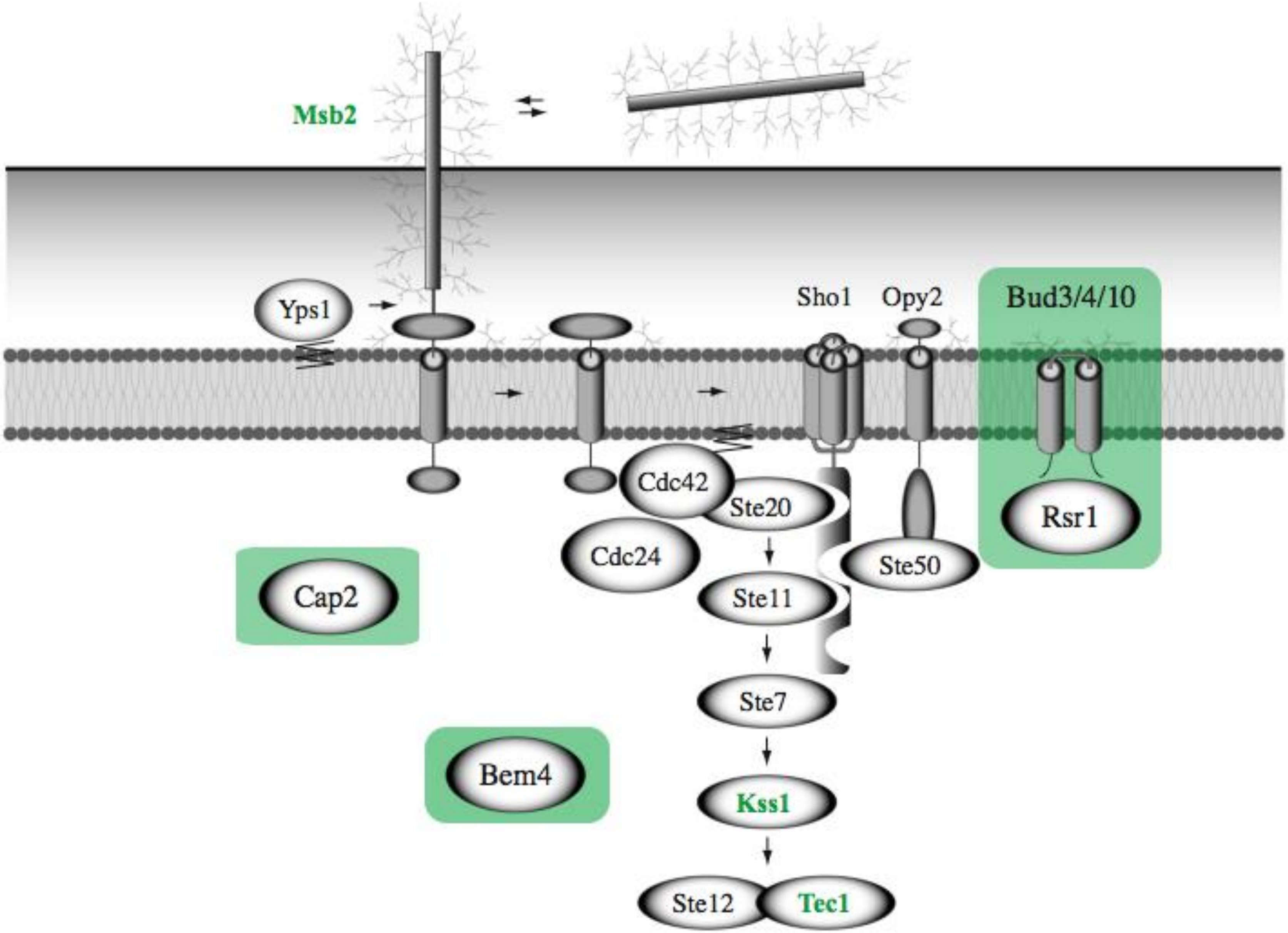
RIM101

SNF1

TOR

Abdullah and Cullen, 2009; Chavel *et al*. (IN PRESS)

The signaling mucin Msb2 functions in the filamentous growth pathway

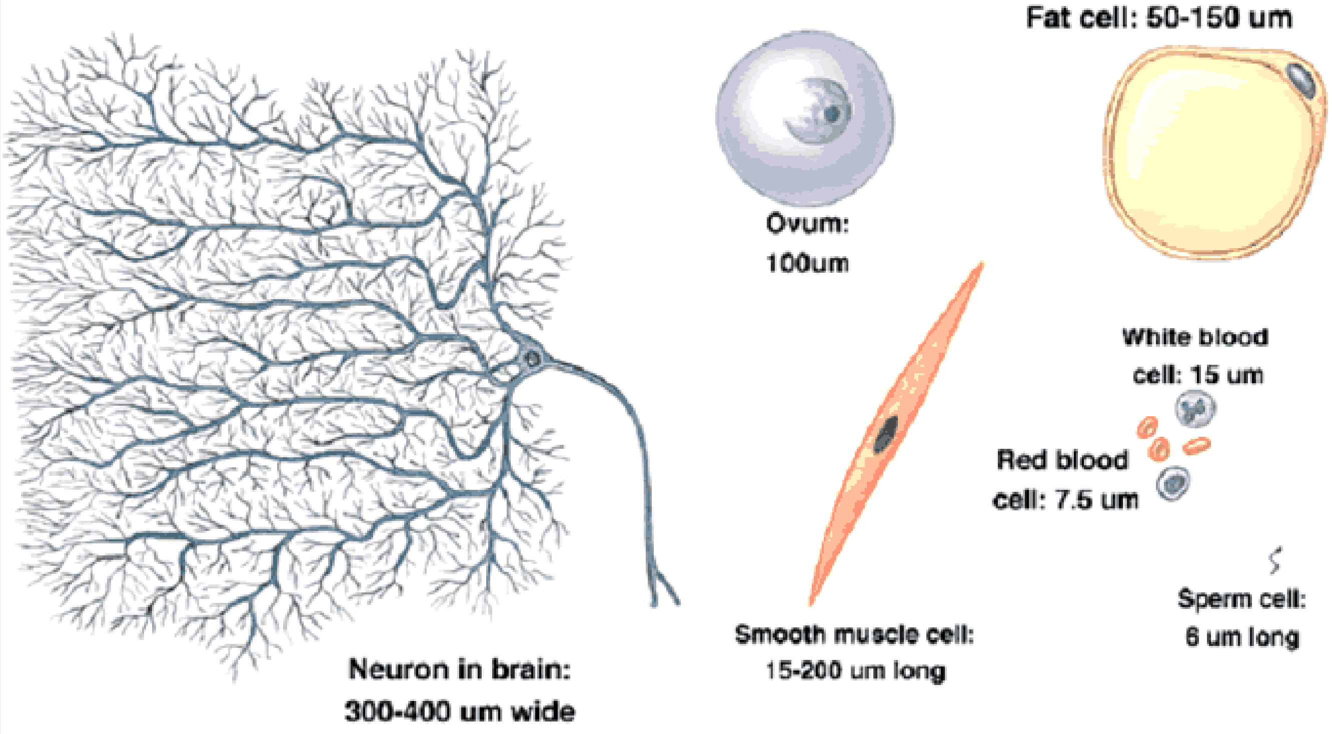


How does a MAPK pathway influence polarity?

Cell differentiation

From 1 zygote to 200 different types of cells

Mechanism: differential gene activation allows creation of specialized cells



Stem cells

Blastomeres are nondifferentiated and can give rise to any tissue

Stem cells are set aside and will continue to divide while remaining undifferentiated

-**Tissue-specific**: can give rise to only one tissue

-**Pluripotent**: can give rise to multiple different cell types

-**Totipotent**: can give rise to any cell type

Background information

- cell fate → what a cell normally develops into
- fate map → marking cells in the embryo ; shows where the tissues of each germ layer normally come from
- Note: having a particular fate does not imply a cell’s full potential

Cell Fate and Morphogen gradient

- *Morphogen*= secreted signaling molecules that organize surrounding cells into patterns
- *Morphogen gradients*= mechanism that one part of embryo can determine location, differentiation, and fate of cells; leading to different cell types

Pattern Formation

In the early stages of pattern formation, two perpendicular axes are established

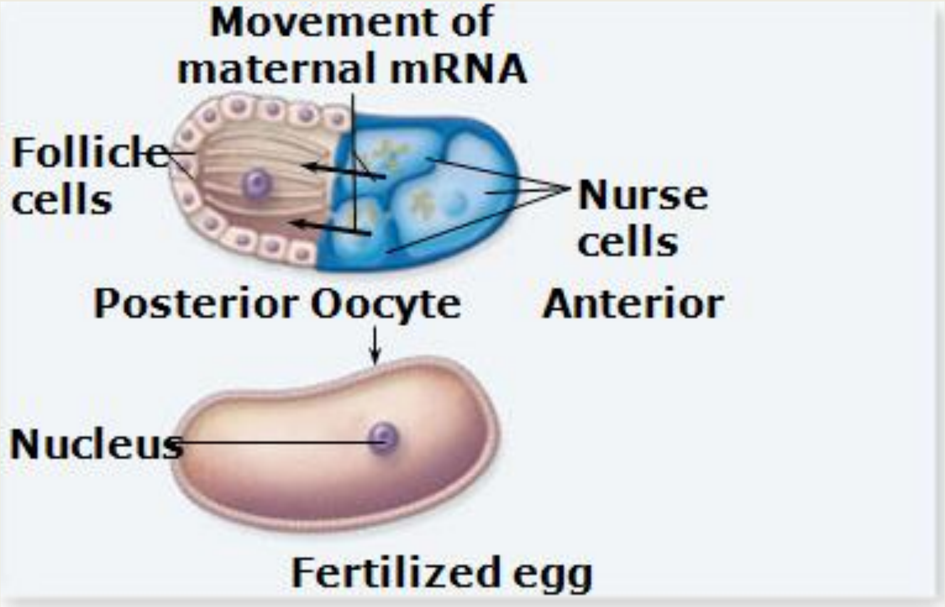
-Anterior/posterior (A/P, head-to-tail) axis

-Dorsal/ventral (D/V, back-to-front) axis

Polarity refers to the acquisition of axial differences in developing structures

Position information leads to changes in gene activity, and thus cells adopt a fate appropriate for their location

Drosophila Embryogenesis

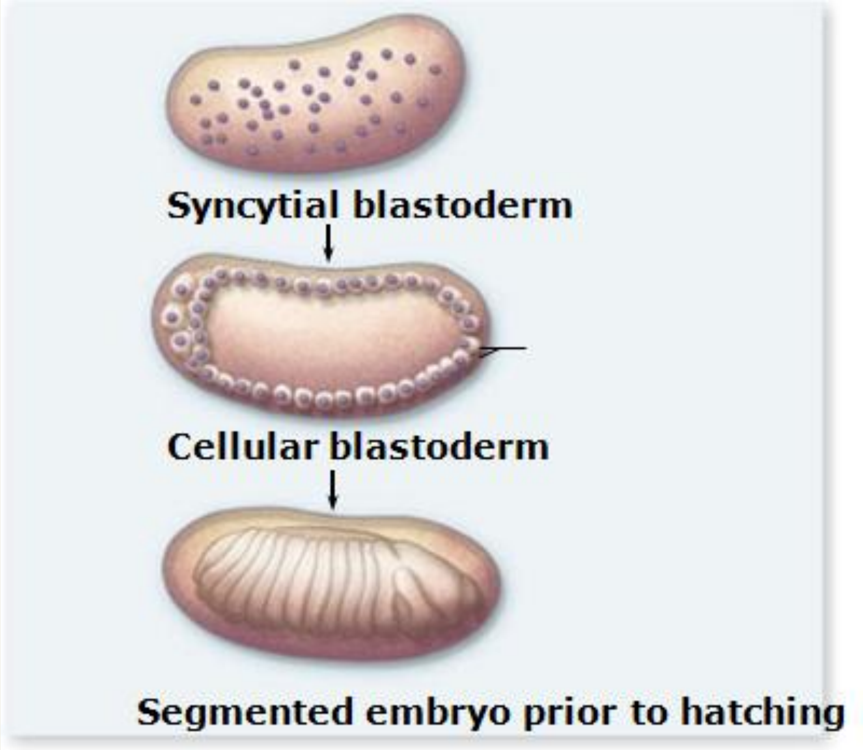


Before fertilization, specialized **nurse cells** move maternal mRNAs into maturing oocyte

-These mRNA will initiate a cascade of gene activations following *fertilization*

Embryonic nuclei do not begin to function until approximately 10 nuclear divisions later

Drosophila Embryogenesis



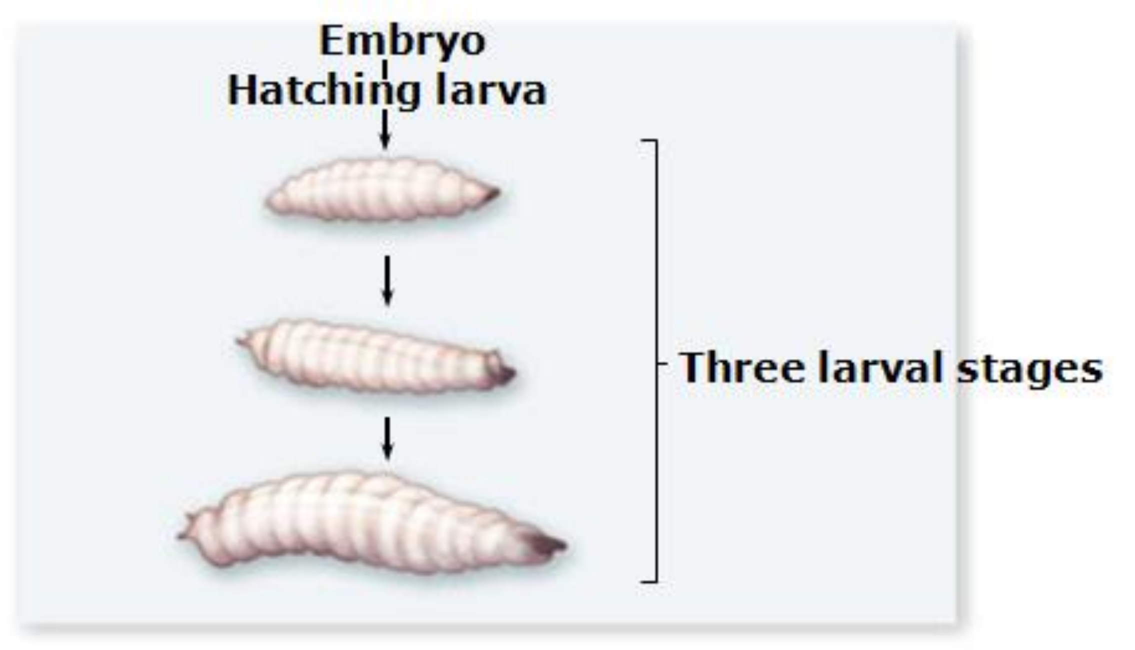
After fertilization, 12 rounds of nuclear division without cytokinesis produces a **syncytial blastoderm**

-4000 nuclei in a single cytoplasm

Membranes grow between the nuclei forming the **cellular blastoderm**

Within a day of fertilization, a segmented, tubular body is formed

Drosophila Embryogenesis



Drosophila produces two body forms

- -**Larva** – Tubular eating machine
- -Adult – Flying sex machine axes are established

Embryogenesis is the formation of a larva from a fertilized egg

Metamorphosis is the passage from one body form to another

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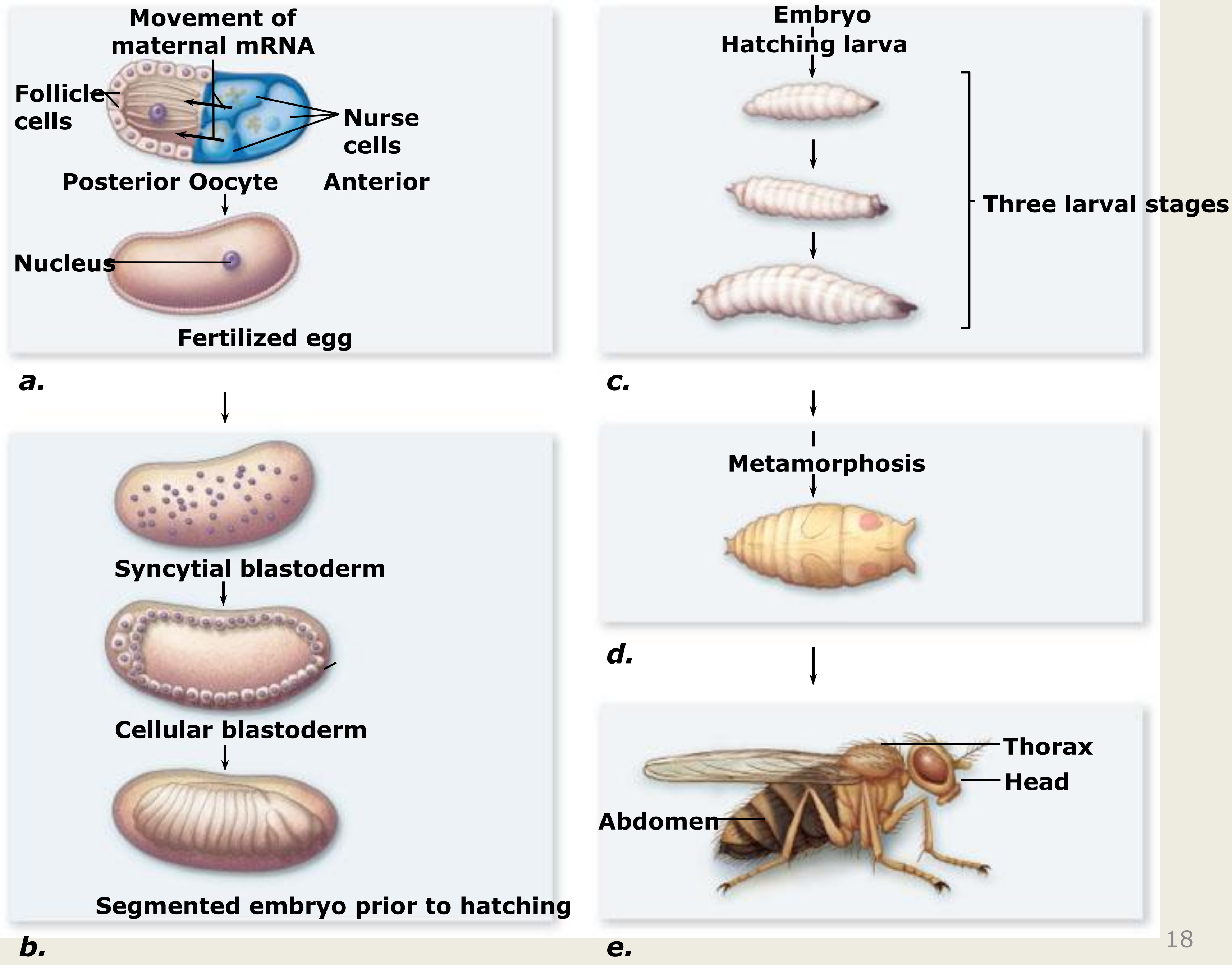

Drosophila Embryogenesis

Nüsslein-Volhard and Wieschaus elucidated how the segmentation pattern is formed

-Earned the 1995 Nobel Prize

Two different genetic pathways control the establishment of the A/P and D/V polarity

-Both involve gradients of **morphogens**

-Soluble signal molecules that can specify different cell fates along an axis

Establishment of the A/P axis

Nurse cells secrete maternally produced *bicoid* and *nanos* mRNAs into the oocyte

-Differentially transported by microtubules to opposite poles of the oocyte

-*bicoid* mRNA to the future anterior pole

-*nanos* mRNA to the future posterior pole

-After fertilization, translation will create opposing gradients of **Bicoid** and **Nanos** proteins

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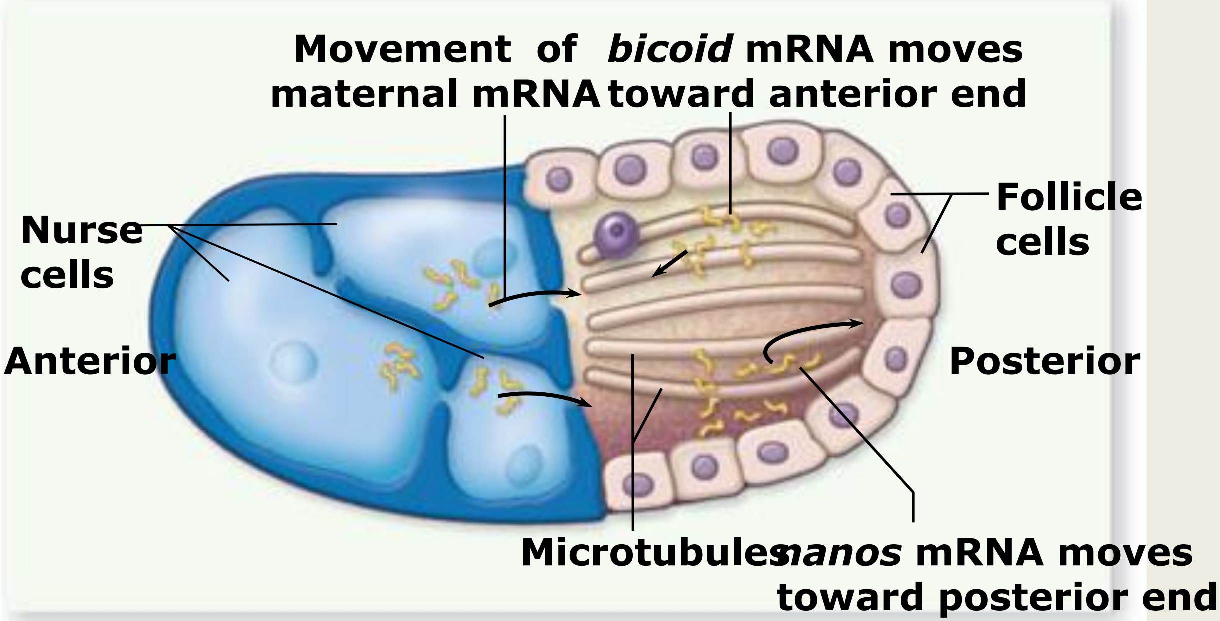
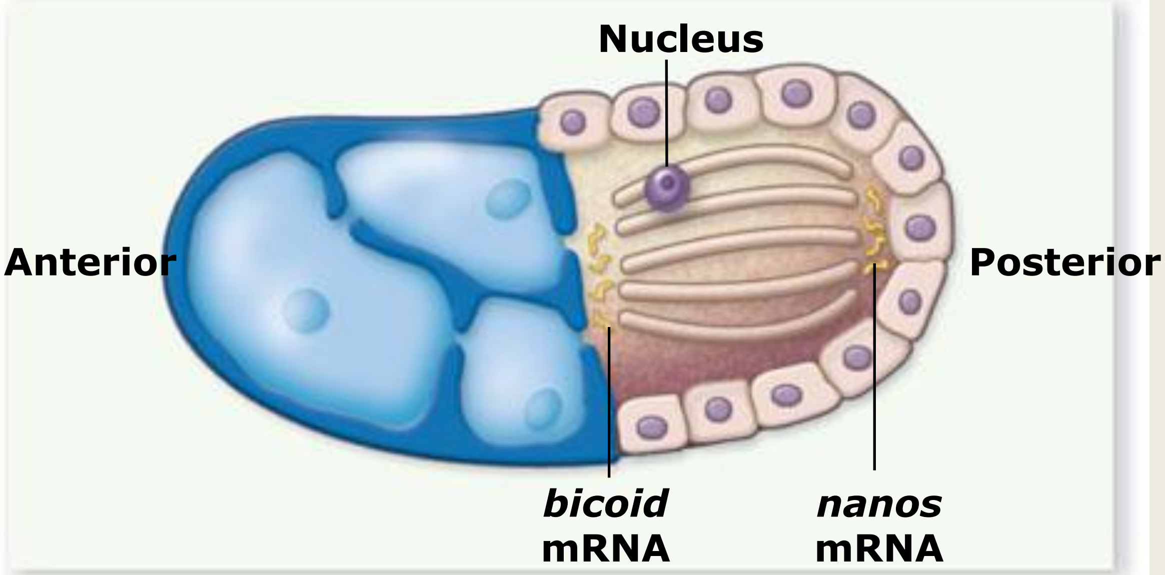

Movement of maternal mRNA
bicoid mRNA moves toward anterior end
Nurse cells
Follicle cells
Anterior
Posterior
Microtubules
nanos mRNA moves toward posterior end

a.



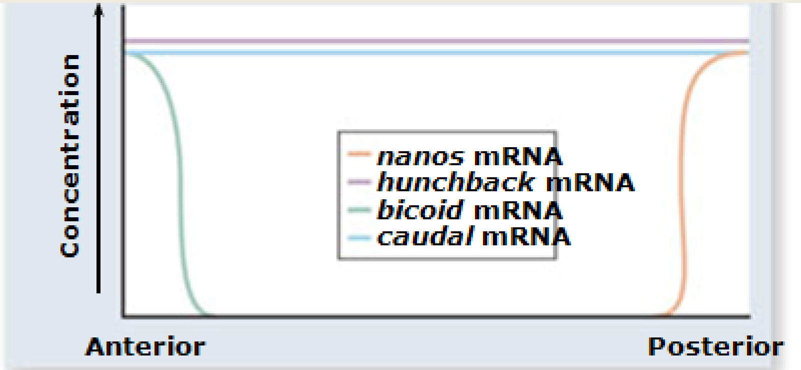
b.

Establishment of the A/P axis

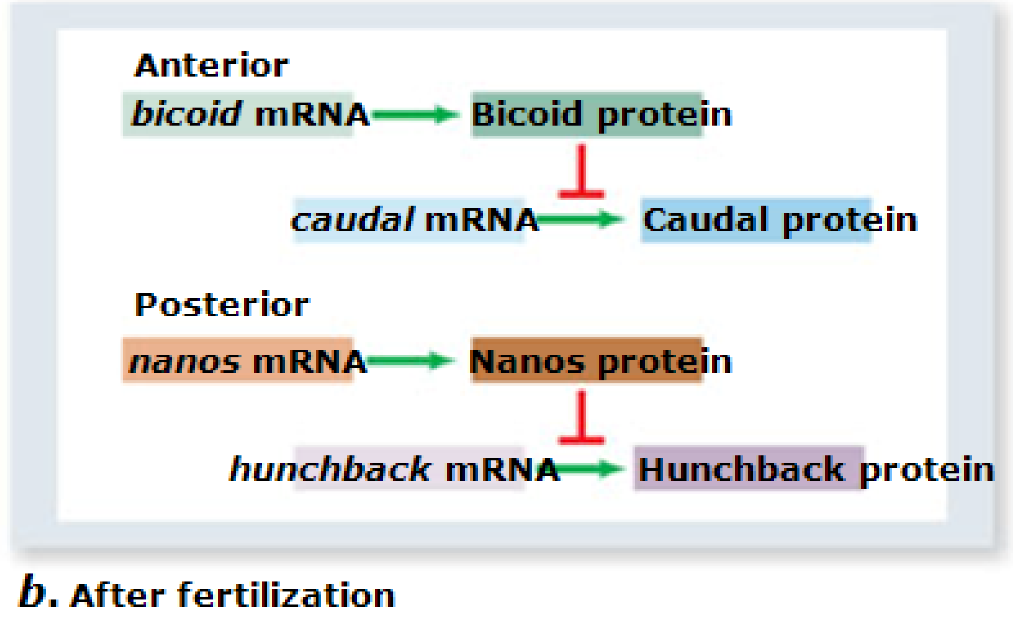
Bicoid and Nanos control translation of two other maternal mRNAs, *hunchback* and *caudal*, that encode transcription factors

-**Hunchback (горбун)** activates anterior structures

-**Caudal (хвостовой)** activates posterior structures



a. Oocyte mRNAs

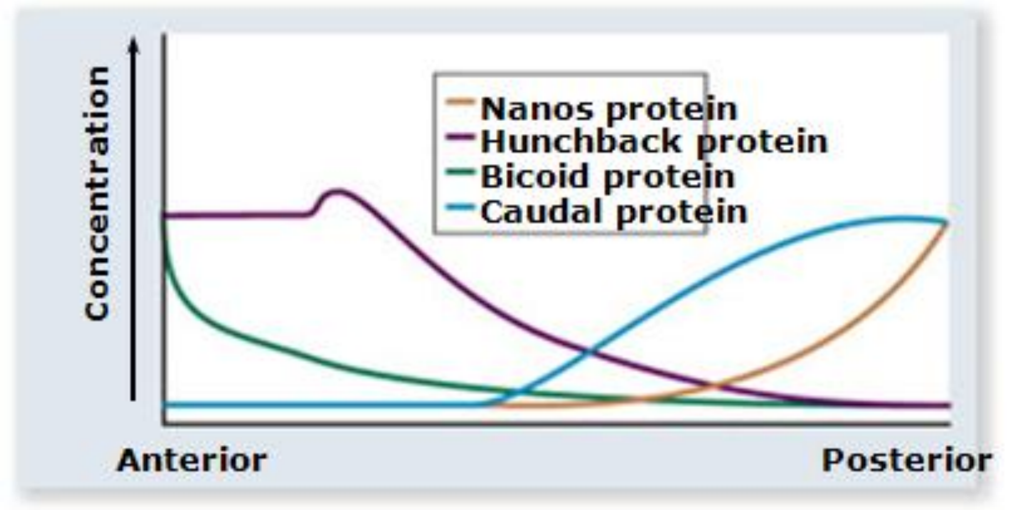


b. After fertilization

The two mRNAs are not evenly distributed

-Bicoid inhibits *caudal* mRNA translation

-Nanos inhibits *hunchback* mRNA translation



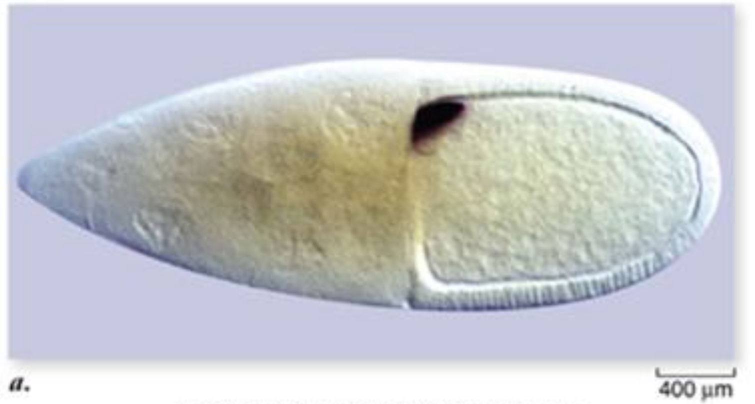
C. Early cleavage embryo proteins

Establishment of the D/V axis

Maternally produced *dorsal* mRNA is placed into the oocyte, Not asymmetrically localized

Oocyte nucleus synthesizes *gurken* (*огурец, нем.*) mRNA, фссumulates in a *crescent* (серповидный, полумесяц) on the future dorsal side of embryo

After fertilization, a series of steps results in selected transport of **Dorsal** into ventral nuclei, thus forming a D/V gradient



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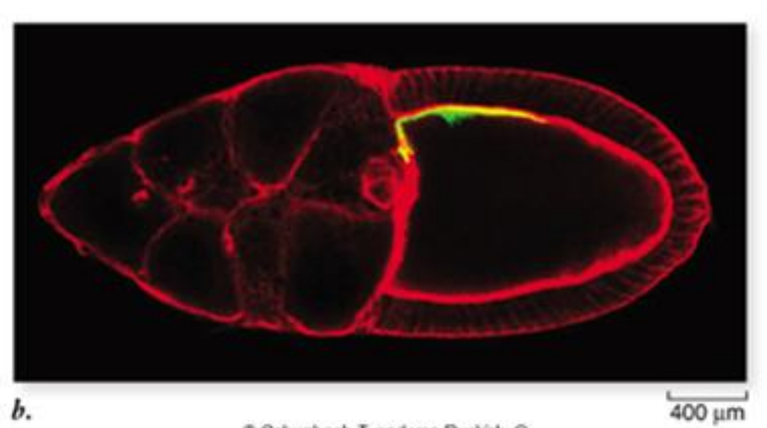

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Production of Body Plan

The body plan is produced by sequential activation of three classes of **segmentation genes**

1. Gap genes

-Map out the coarsest (грубое) subdivision along the A/P axis

-All 9 genes encode transcription factors that activate the next gene class

Production of Body Plan

2. Pair-rule genes

-Divide the embryo into seven zones

-The 8 or more genes encode transcription factors that regulate each other, and activate the next gene class

3. Segment polarity genes

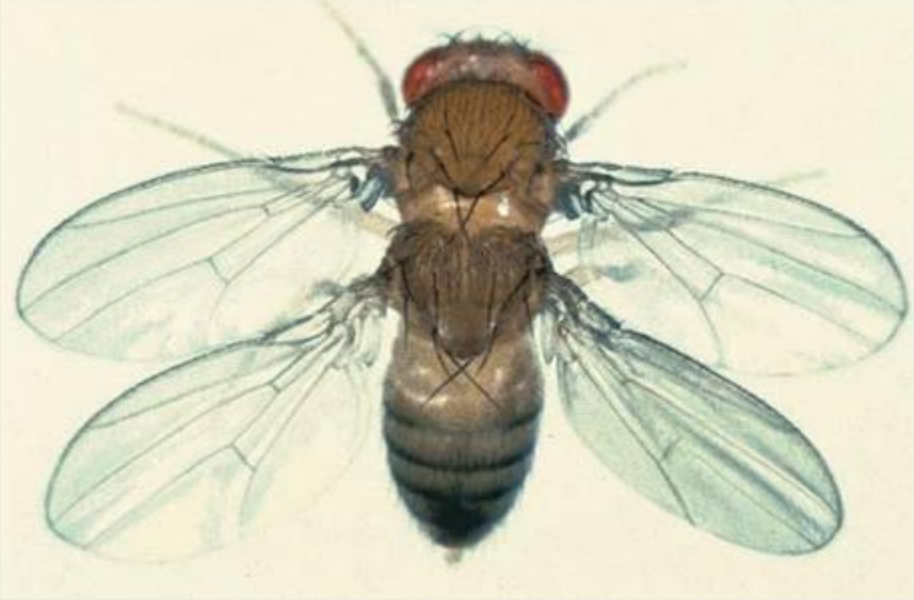
-Finish defining the embryonic segments

Production of Body Plan

Segment identity arises from the action of **homeotic genes**

-Mutations in them lead to the appearance of normal body parts in unusual places

-*Ultrabithorax* mutants produce an extra pair of wings



Production of Body Plan

Homeotic gene complexes

-The **HOM complex** genes of *Drosophila* are grouped into two clusters

- -**Antennapedia complex**, which governs the anterior end of the fly
- -**Bithorax complex**, which governs the posterior end of the fly

-Interestingly, the order of genes mirrors the order of the body parts they control

Production of Body Plan

Homeotic gene complexes

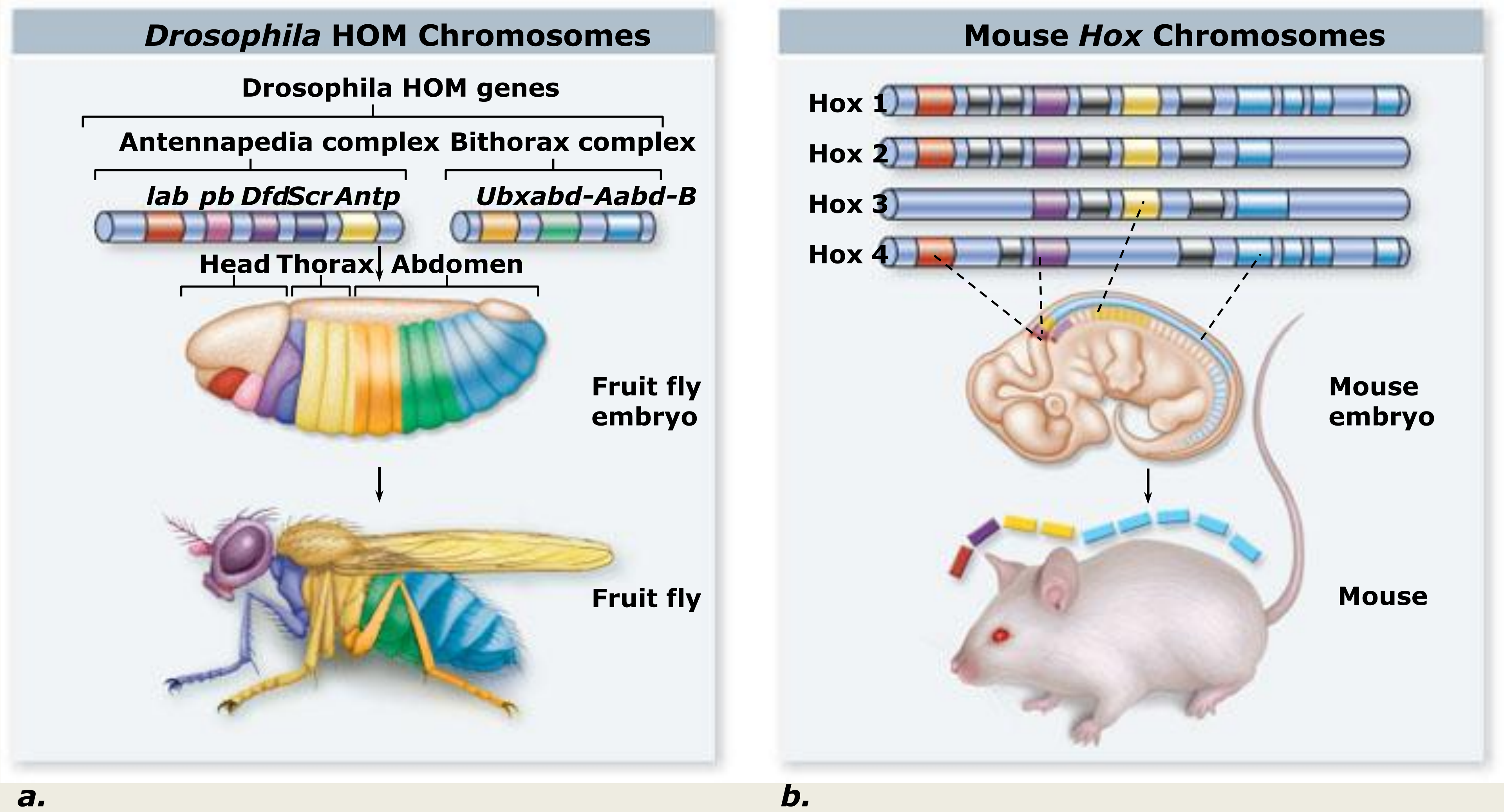
-All of these genes contain a conserved 180-base sequence, the **homeobox**

-Encodes a 60-amino acid DNA-binding domain, the **homeodomain**

-Homeobox-containing genes are termed ***Hox* genes**

-Vertebrates have 4 *Hox* gene clusters

Production of Body Plan



Pattern Formation in Plants

The predominant homeotic gene family in plants is the **MADS-box genes**

-Found in most eukaryotic organisms, although in much higher numbers in plants

MADS-box genes encode transcriptional regulators, which control various processes:

-Transition from vegetative to reproductive growth, root development and floral organ identity

Morphogenesis

Morphogenesis is the formation of ordered form and structure

-Animals achieve it through changes in:

- Cell division
- Cell shape and size
- Cell death
- Cell migration

-Plants use these except for cell migration

Morphogenesis

Cell division

-The orientation of the mitotic spindle determines the plane of cell division in eukaryotic cells

-If spindle is centrally located, two equal-sized daughter cells will result

-If spindle is off to one side, two unequal daughter cells will result

Morphogenesis

Cell shape and size

-In animals, cell differentiation is accomplished by profound changes in cell size and shape

  -Nerve cells develop long processes called axons

  -Skeletal muscles cells are large and multinucleated

Morphogenesis

Cell death

- -**Necrosis** is accidental cell death
- -**Apoptosis** is programmed cell death
  - -Is required for normal development in all animals
  - -“Death program” pathway consists of:
    - -Activator, inhibitor and apoptotic protease

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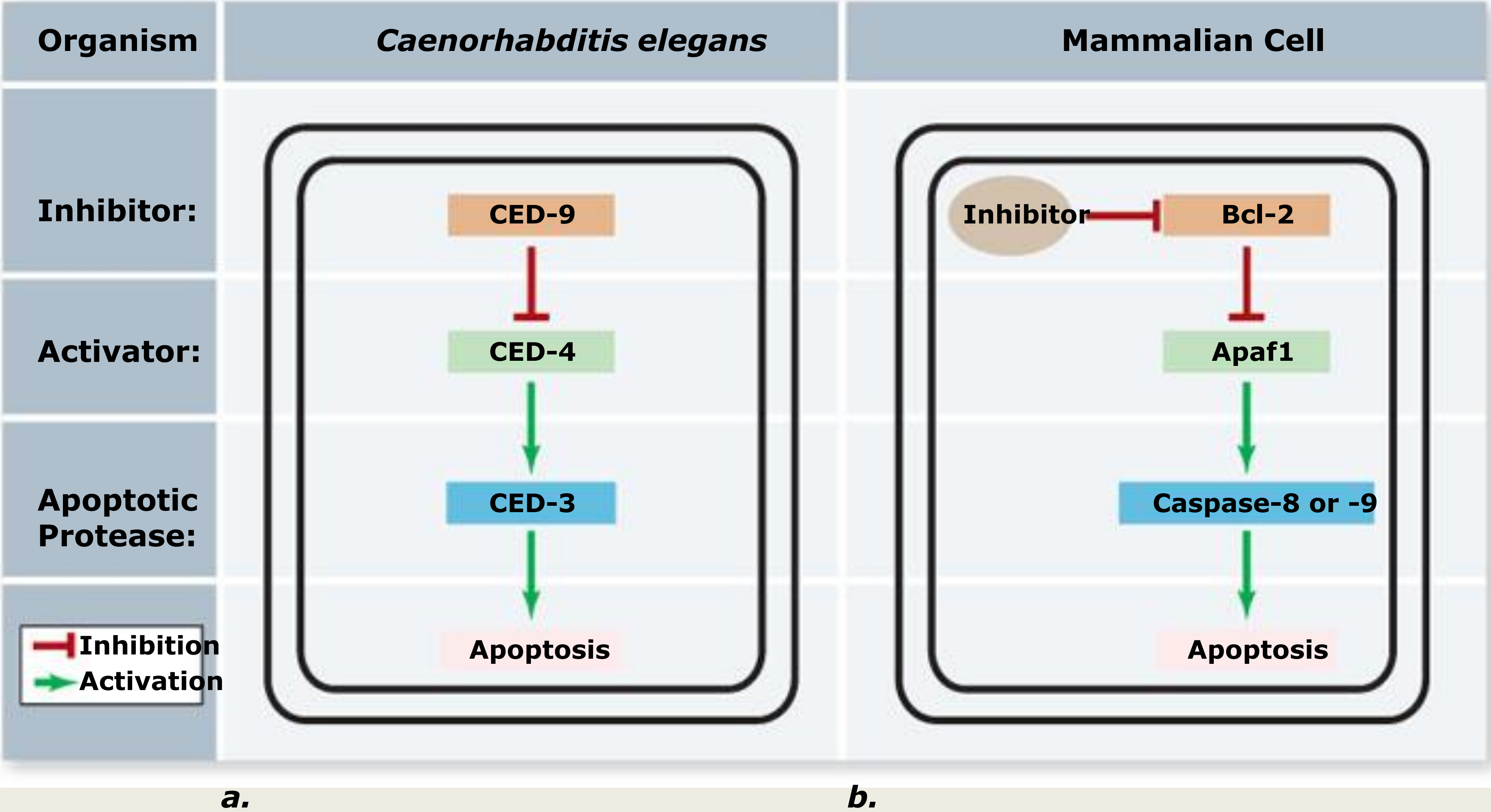

Organism	***Caenorhabditis elegans***	**Mammalian Cell**
Inhibitor:	**CED-9**	**Inhibitor** ⊣ **Bcl-2**
Activator:	**CED-4**	**Apaf1**
Apoptotic Protease:	**CED-3**	**Caspase-8 or -9**
⊣ **Inhibition** → **Activation**	**Apoptosis**	**Apoptosis**

a. **b.**

Morphogenesis

Cell migration

-Cell movement involves both adhesion and loss of adhesion between cells and substrate

-Cell-to-cell interactions are often mediated through **cadherins**

-Cell-to-substrate interactions often involve complexes between **integrins** and the **extracellular matrix (ECM)**

Development of Seed Plants

Plant development occurs in five main stages:

1. Early embryonic cell division

- First division is off-center
  - Smaller cell divides to form the embryo
  - Larger cell divides to form **suspensor**
    - Cells near it ultimately form the root
    - Cells on the other end, form the shoot

Development of Seed Plants

2. Embryonic tissue formation

- Three basic tissues differentiate:
  - Epidermal, ground and vascular

3. Seed formation

- 1-2 cotyledons form
- Development is arrested

4. Seed germination

- Development resumes
- Roots extend down, and shoots up

Development of Seed Plants

5. Meristematic development and morphogenesis

-Apical meristems at the root and shoot tips generate a large numbers of cells

-Form leaves, flowers and all other components of the mature plant

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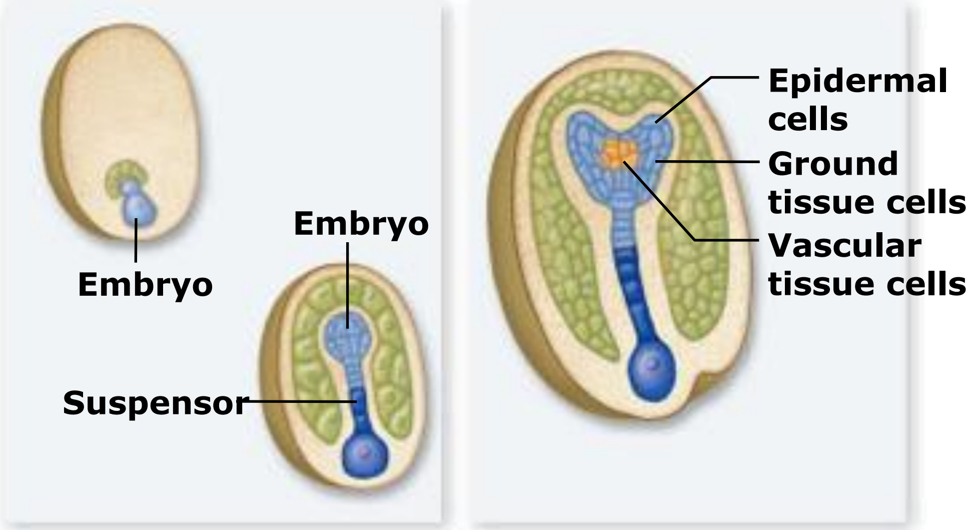
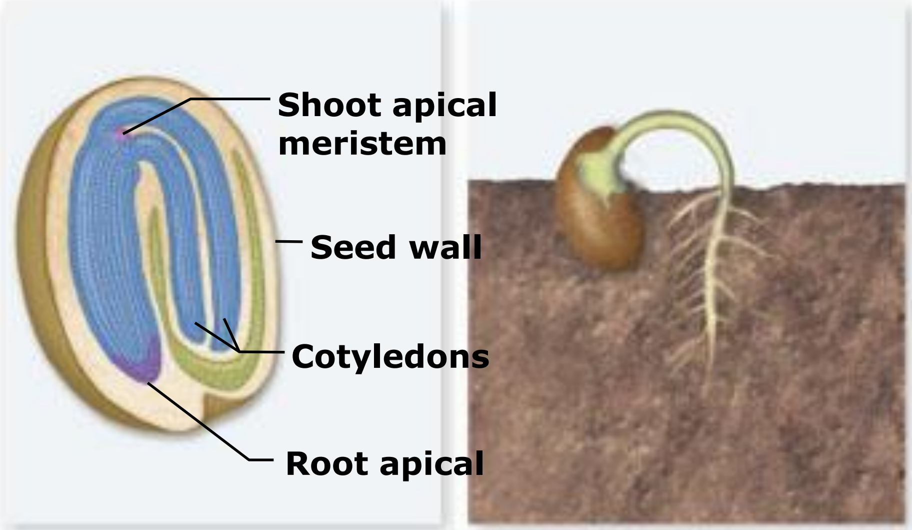


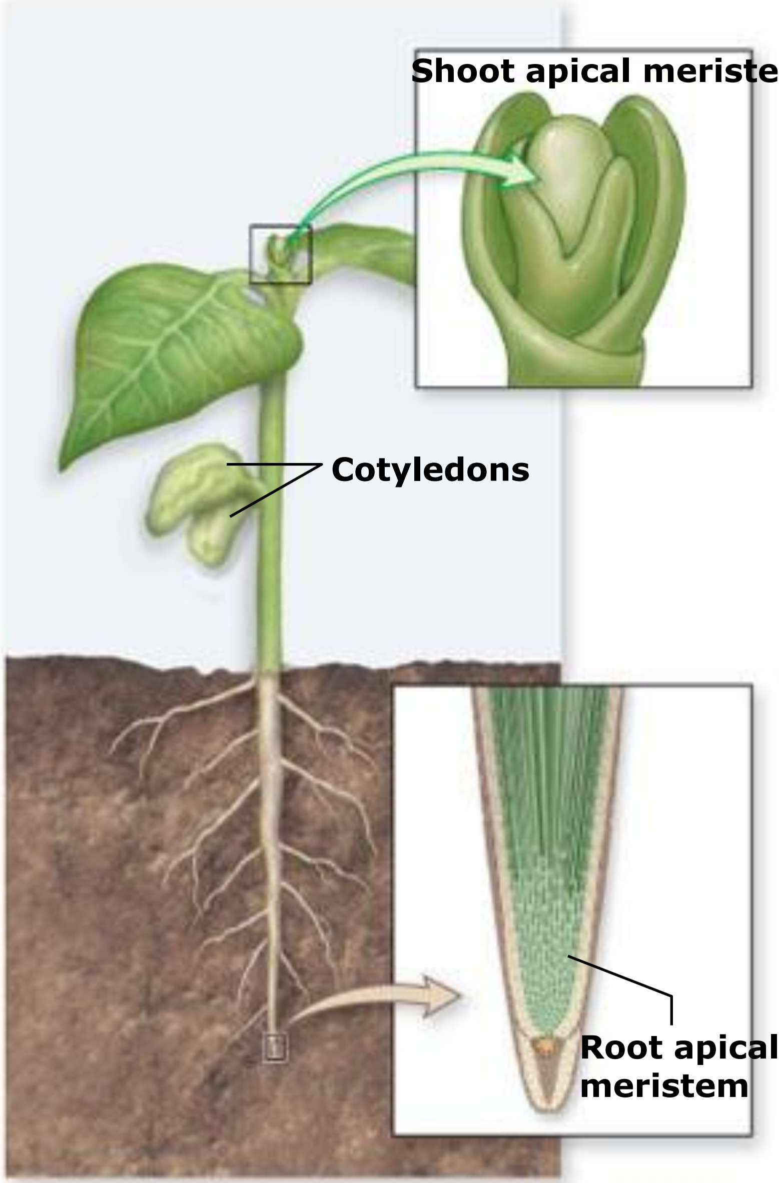
***a*. Early cell division**

***b*. Tissue formation**



***c*. Seed formation meristem**

***d*. Germination**



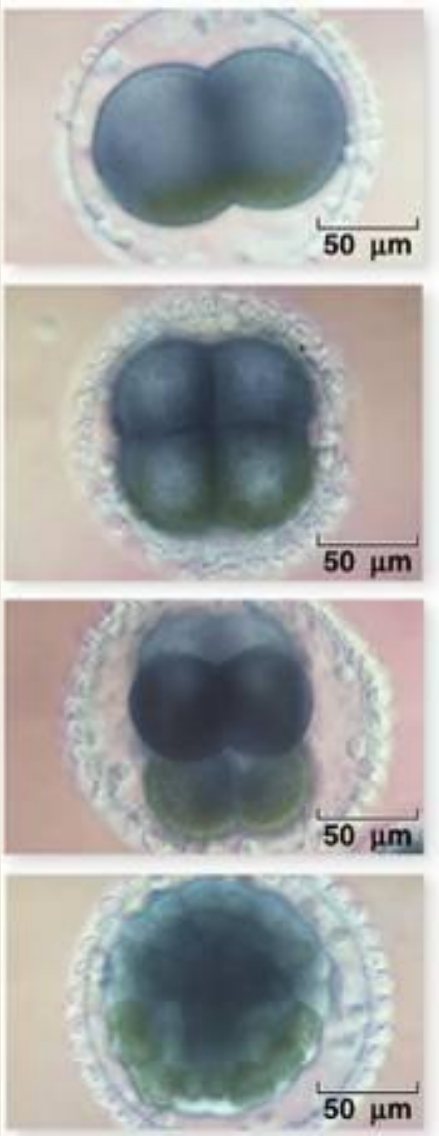
***e*. Meristematic development and morphog**

Cell Differentiation

Cytoplasmic determinants

-Female parent provides egg with *macho-1* mRNA

-Encodes a transcription factor that can activate expression of muscle-specific genes



Cell Differentiation

Induction is the change in the fate of a cell due to interaction with an adjacent cell

If cells of a frog embryo are separated:

-One pole (“animal pole”) forms ectoderm

-Other pole (“vegetal pole”) forms endoderm

-No mesoderm is formed

If the two pole cells are placed side-by-side, some animal-pole cells form the mesoderm

Cell Differentiation

Another example of induction is the formation of notochord and mesenchyme in tunicates

-Arise from mesodermal cells that form at the vegetal margin of 32-cell stage embryo

-Cells receive a chemical signal from underlying endodermal cells

    -Anterior cells differentiate into notochord

    -Posterior cells differentiate into mesenchyme

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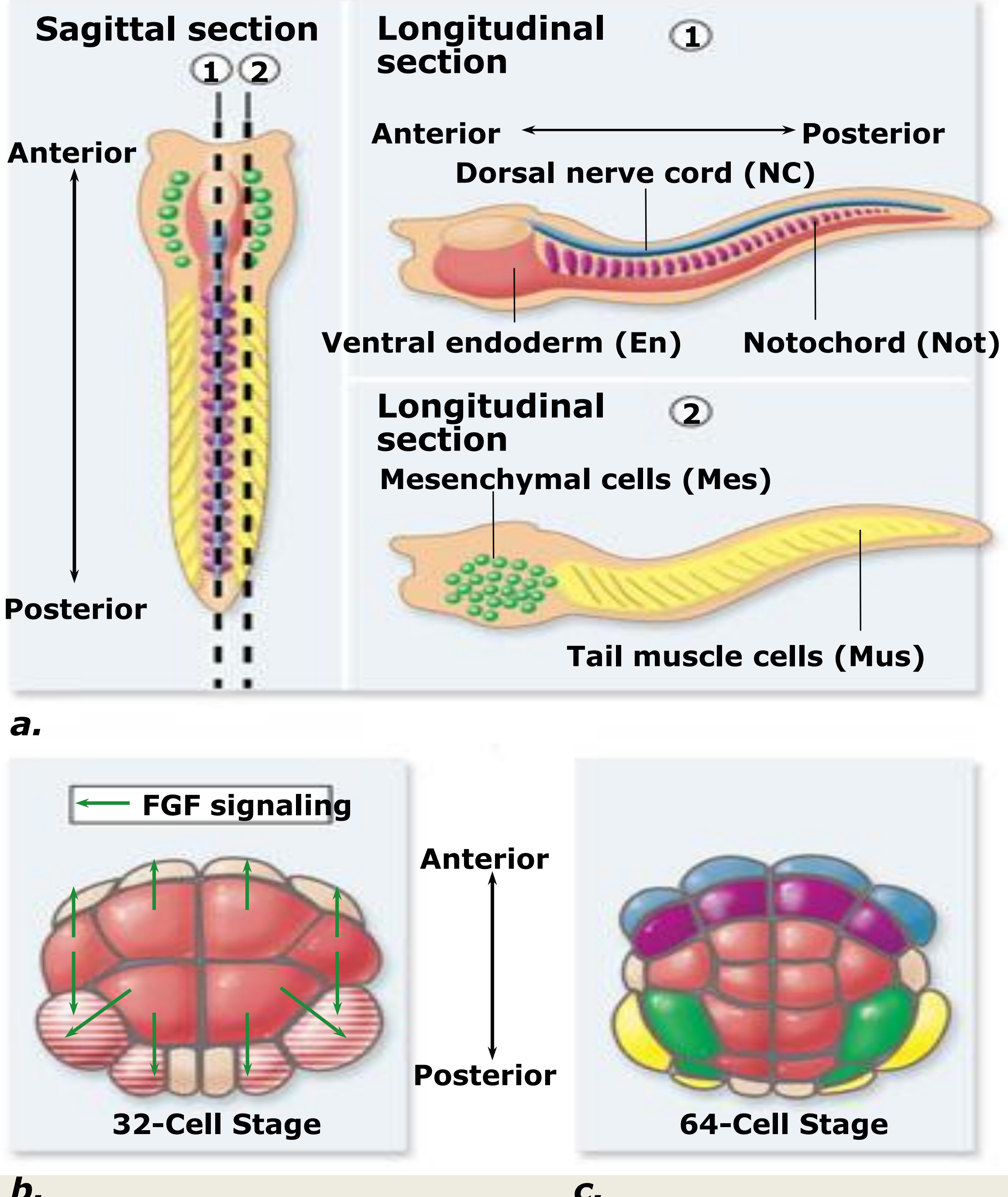


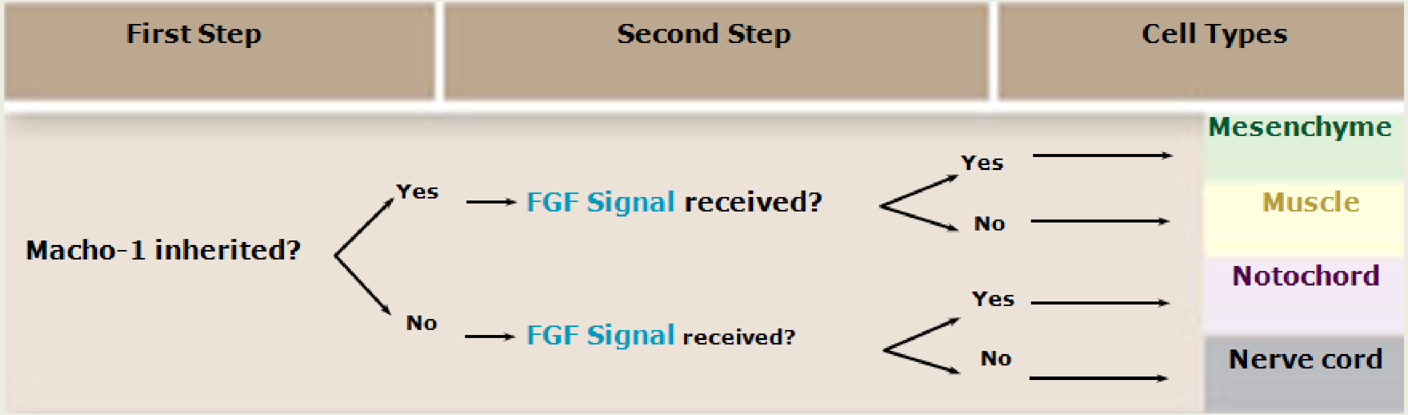
Cell Differentiation

The chemical signal is a **fibroblast growth factor (FGF)** molecule

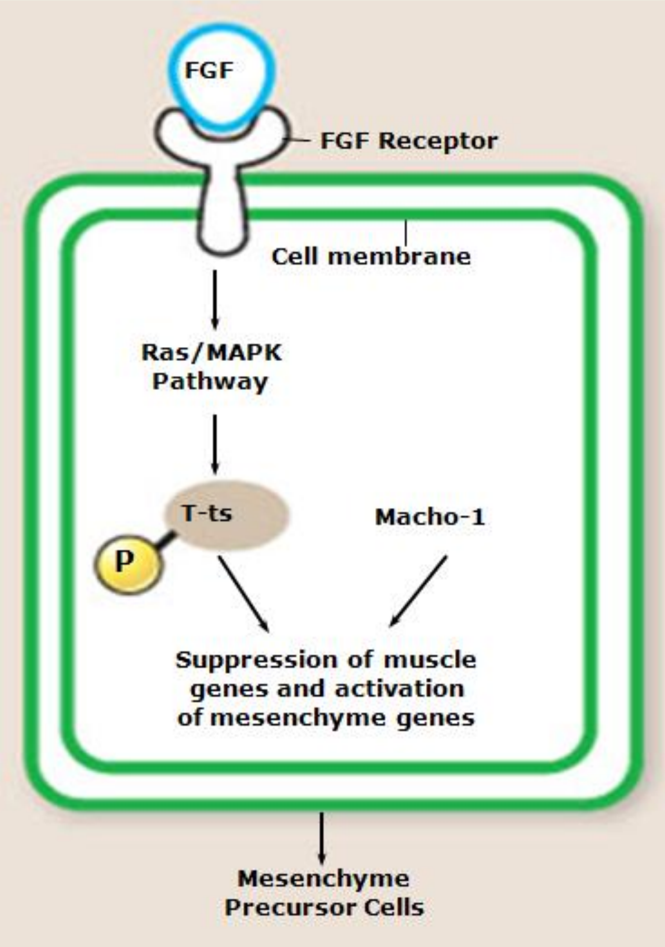
-The FGF receptor is a tyrosine kinase that activates a MAP kinase cascade

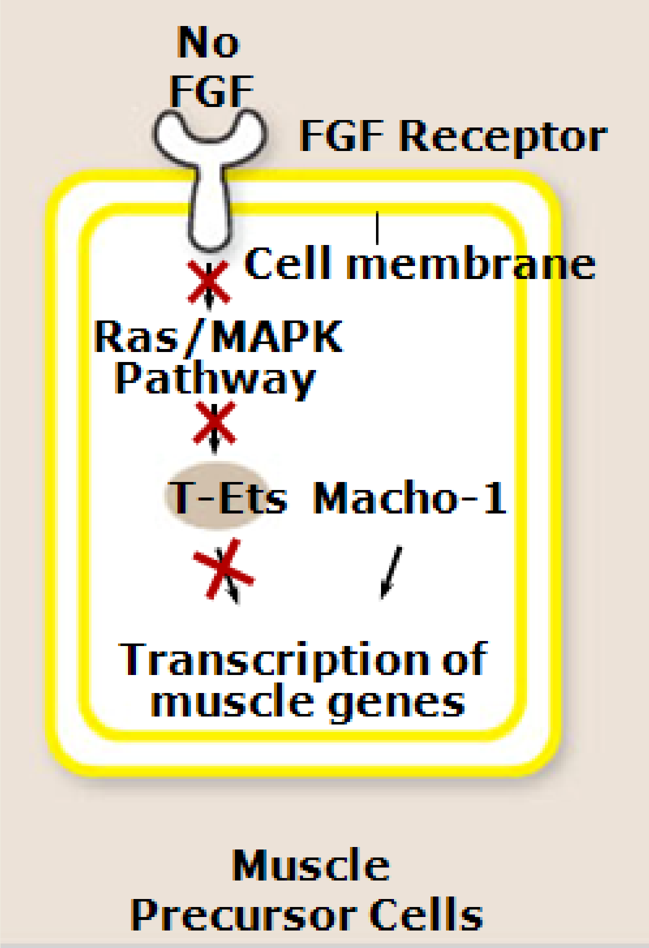
-Produces a transcription factor that triggers differentiation

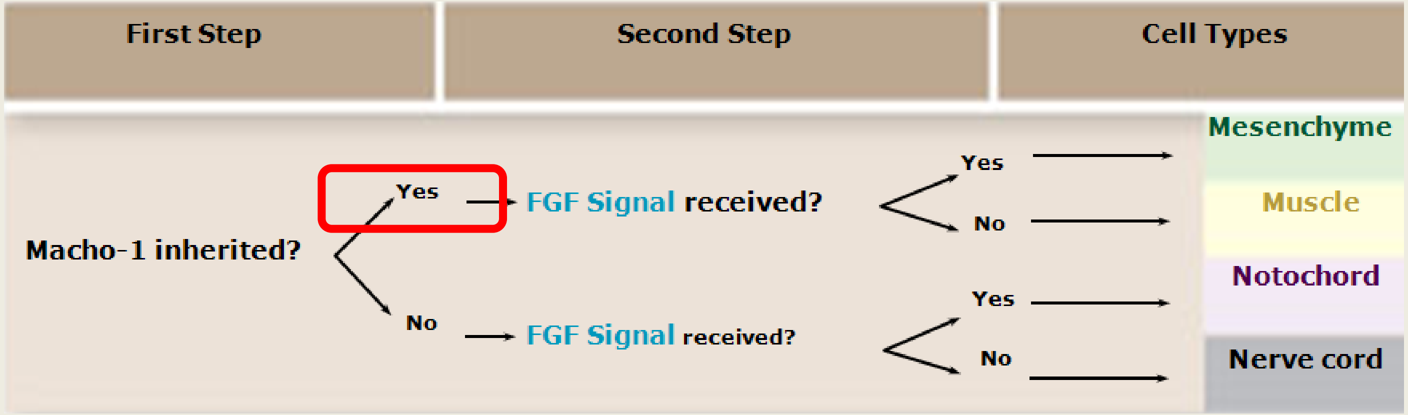
Thus, the combination of *macho-1* and FGF signaling leads to four different cell types



First Step	Second Step	Cell Types
Macho-1 inherited? Yes →	FGF Signal received? Yes →	Mesenchyme
	No →	Muscle
Macho-1 inherited? No →	FGF Signal received? Yes →	Notochord
	No →	Nerve cord


FGF
FGF Receptor
Cell membrane
Ras/MAPK Pathway
T-ts
P
Macho-1
Suppression of muscle genes and activation of mesenchyme genes
Mesenchyme Precursor Cells





First Step | Second Step | Cell Types

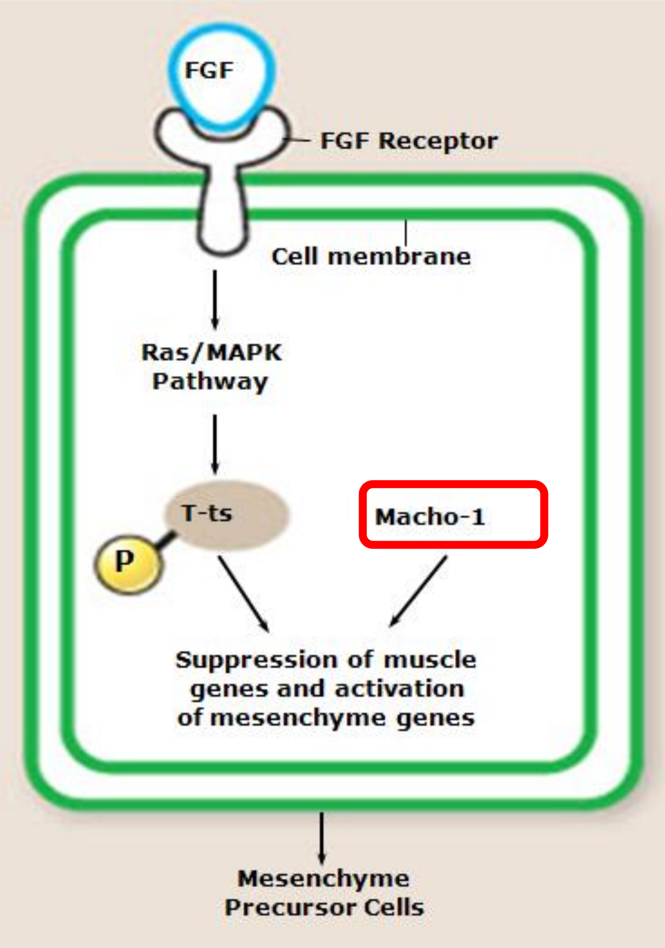
Macho-1 inherited?

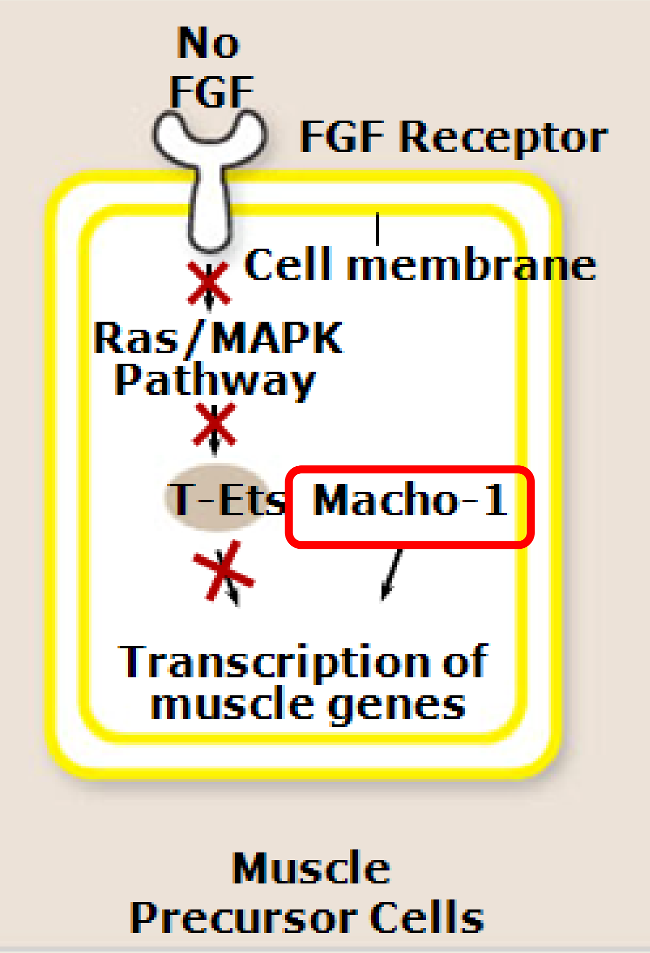
Yes → FGF Signal received?

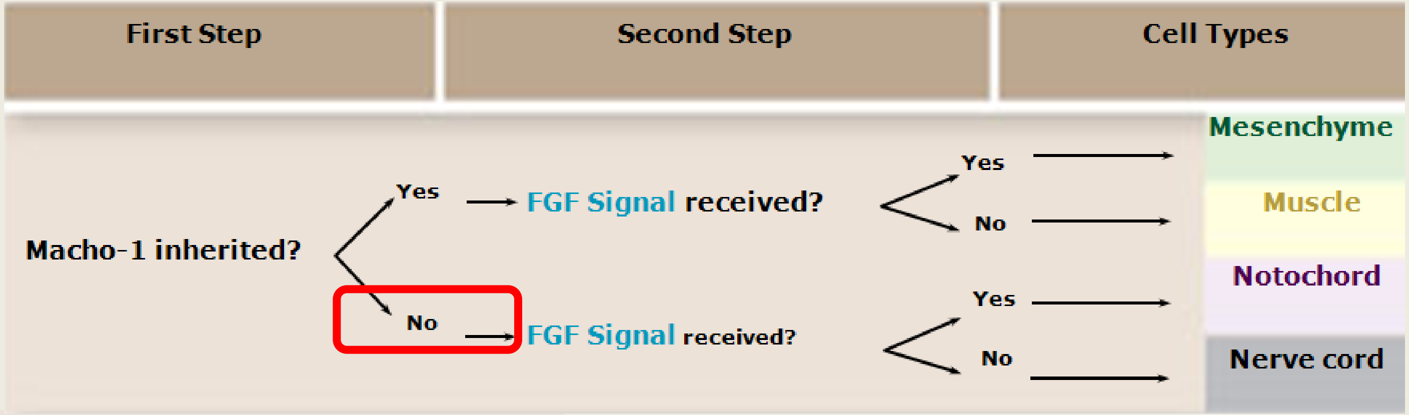
- Yes → Mesenchyme
- No → Muscle

No → FGF Signal received?

- Yes → Notochord
- No → Nerve cord


FGF
FGF Receptor
Cell membrane
Ras/MAPK Pathway
T-ts
P
Macho-1
Suppression of muscle genes and activation of mesenchyme genes
Mesenchyme Precursor Cells





First Step

Second Step

Cell Types

Macho-1 inherited?

Yes → FGF Signal received?

Yes →

No →

No → FGF Signal received?

Yes →

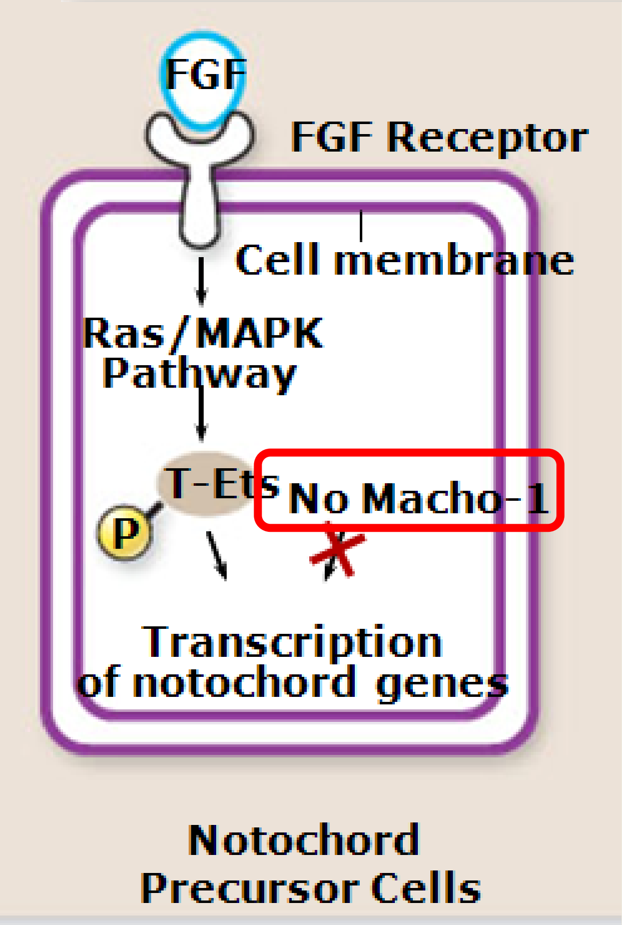
No →

Mesenchyme

Muscle

Notochord

Nerve cord



FGF

FGF Receptor

Cell membrane

Ras/MAPK Pathway

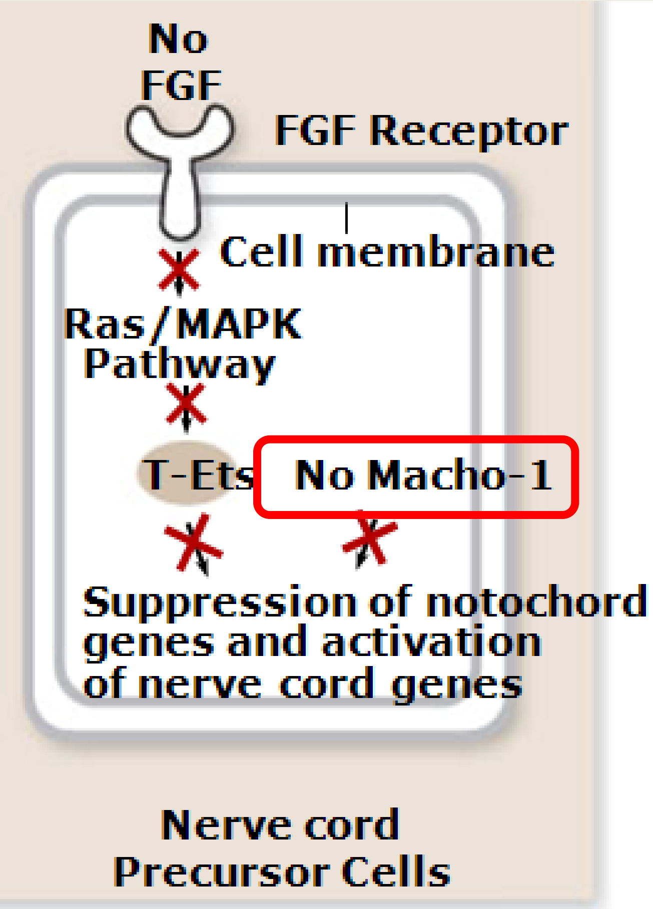
T-Ets

P

No Macho-1

Transcription of notochord genes

Notochord Precursor Cells



No FGF

FGF Receptor

Cell membrane

Ras/MAPK Pathway

T-Ets

No Macho-1

Suppression of notochord genes and activation of nerve cord genes

Nerve cord Precursor Cells

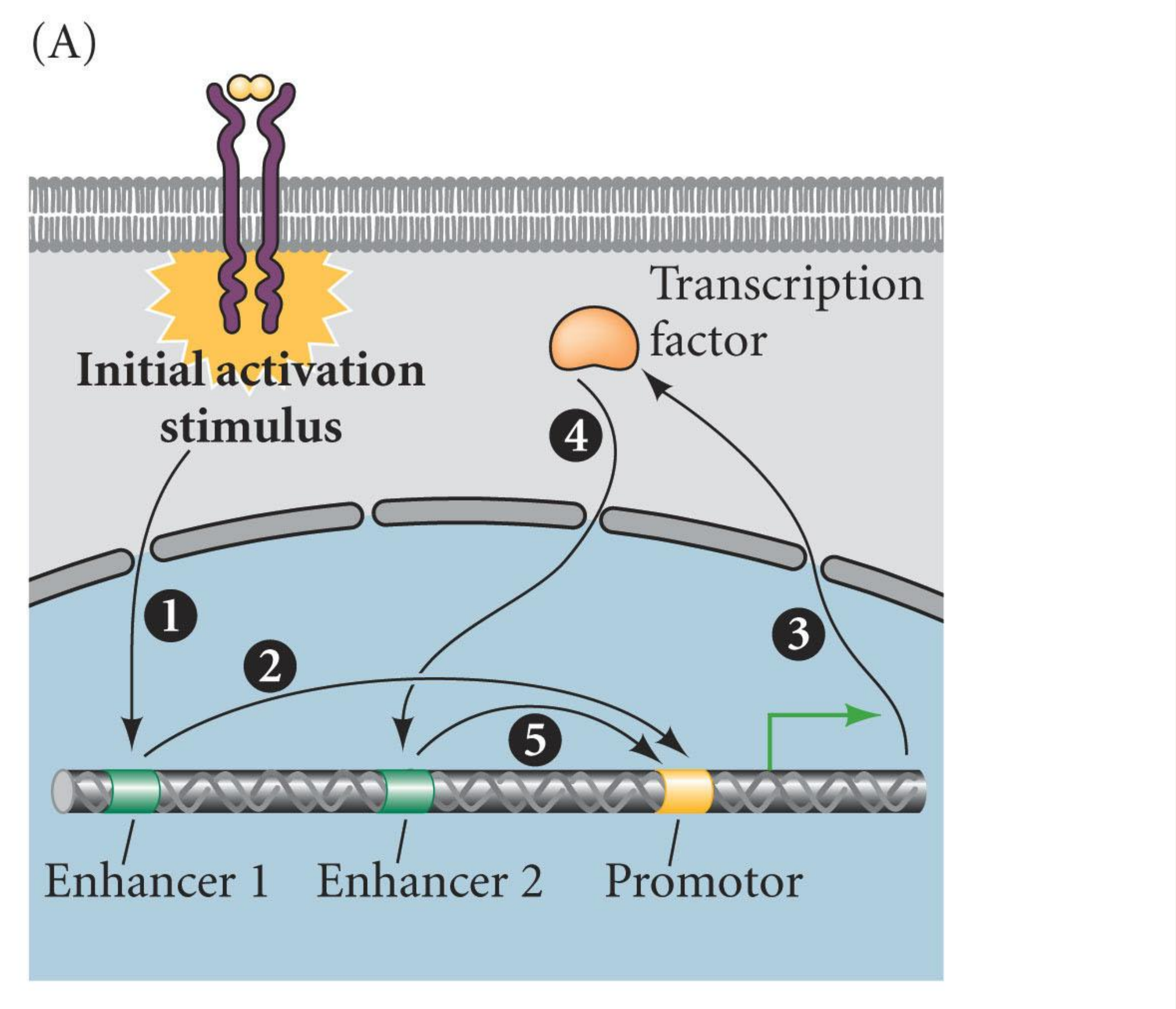
Maintaining Differentiation

Means maintaining gene expression

Four mechanisms

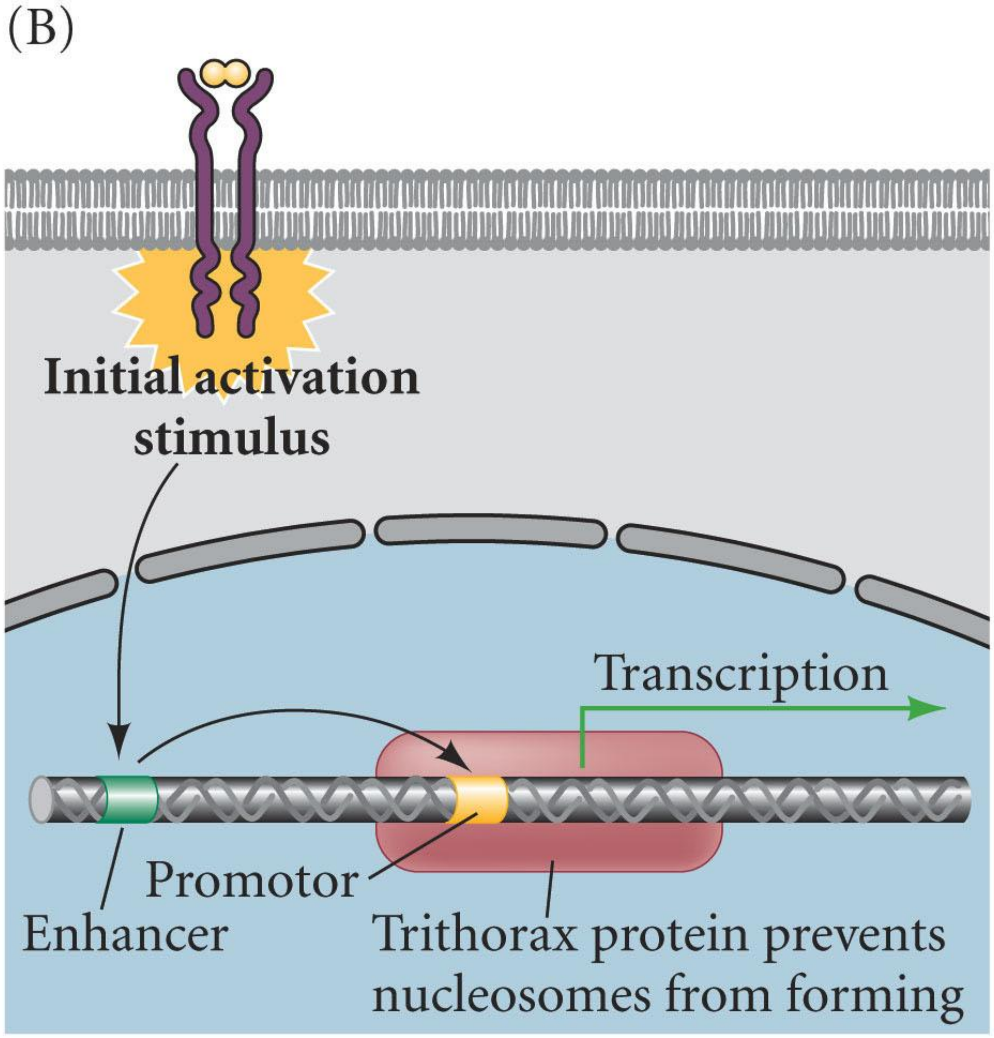
1. Autoinduction by transcription factor
2. Maintain open chromatin structure
3. Autocrine regulation (Community effect)
4. Neighbor-neighbor stimulation with paracrine factors

Four ways of maintaining differentiation after the initial signal has been given (Part 1)



DEVELOPMENTAL BIOLOGY, Eighth Edition, Figure 6.38 (Part 1) © 2006 Sinauer Associates, Inc.

Four ways of maintaining differentiation after the initial signal has been given (Part 2)


(B)
Initial activation stimulus
Transcription
Promotor
Enhancer
Trithorax protein prevents nucleosomes from forming

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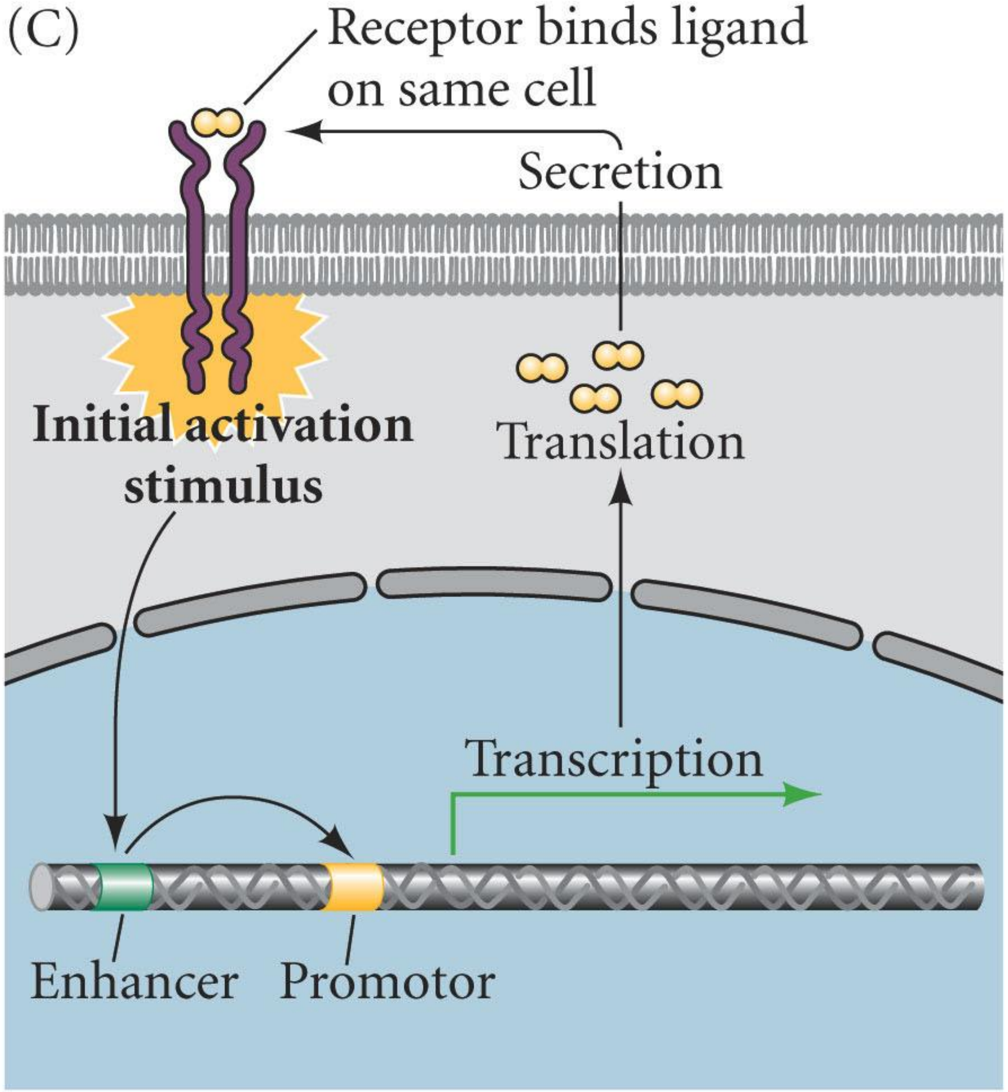
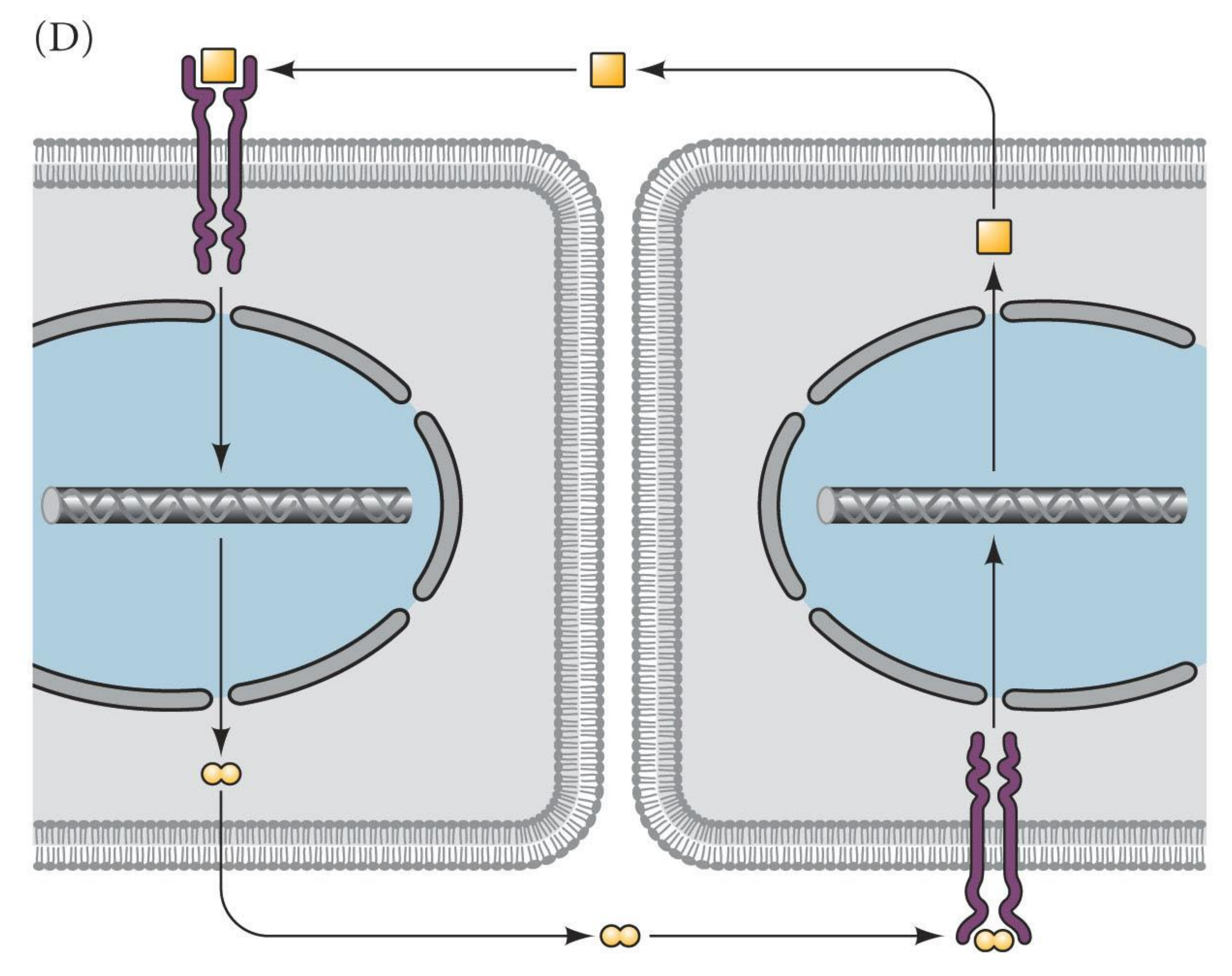
Four ways of maintaining differentiation after the initial signal has been given (Part 3)


(C)
Receptor binds ligand on same cell
Secretion
Initial activation stimulus
Translation
Transcription
Enhancer
Promotor

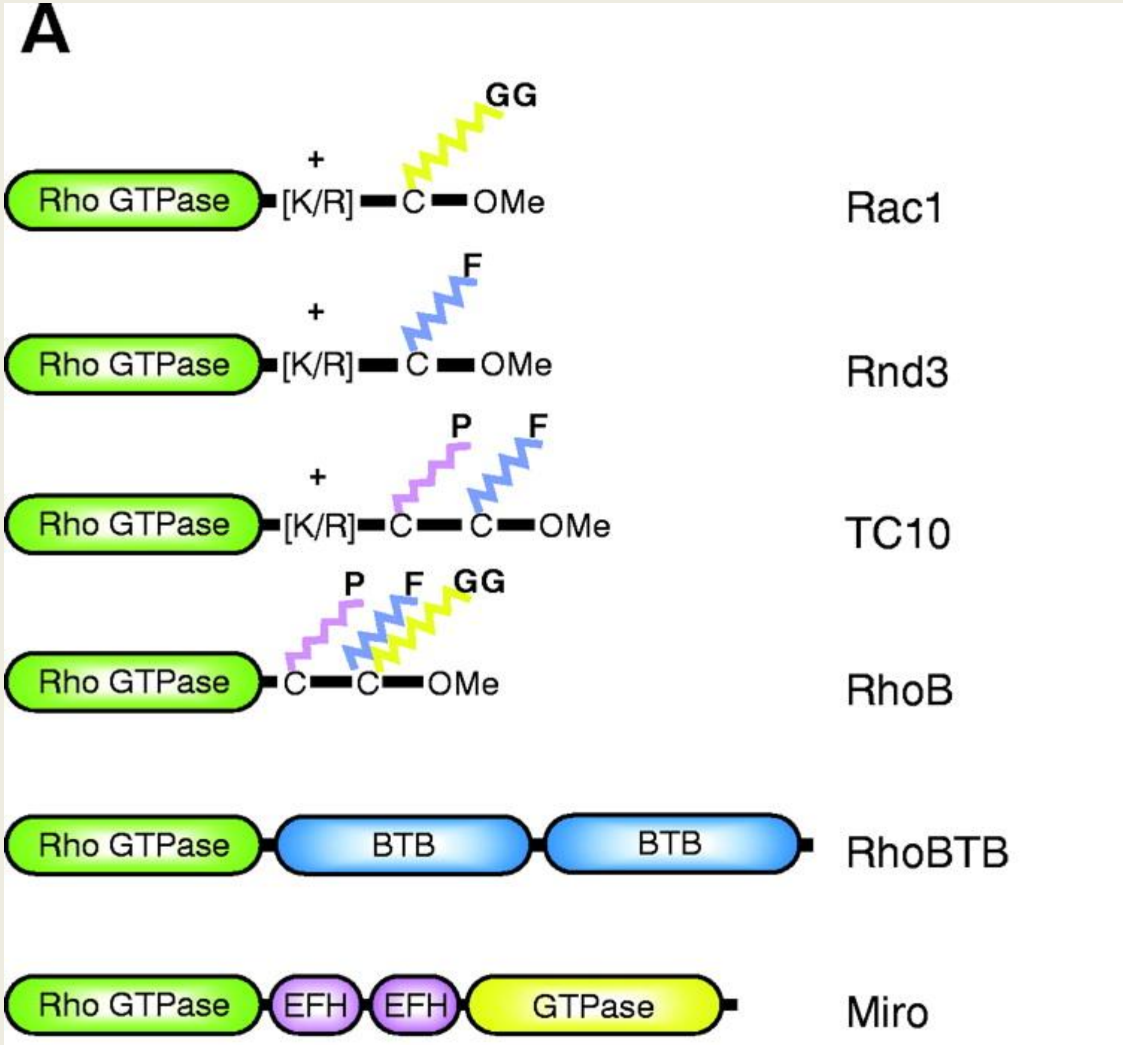
DEVELOPMENTAL BIOLOGY, *Eighth Edition*, Figure 6.38 (Part 3) © 2006 Sinauer Associates, Inc.

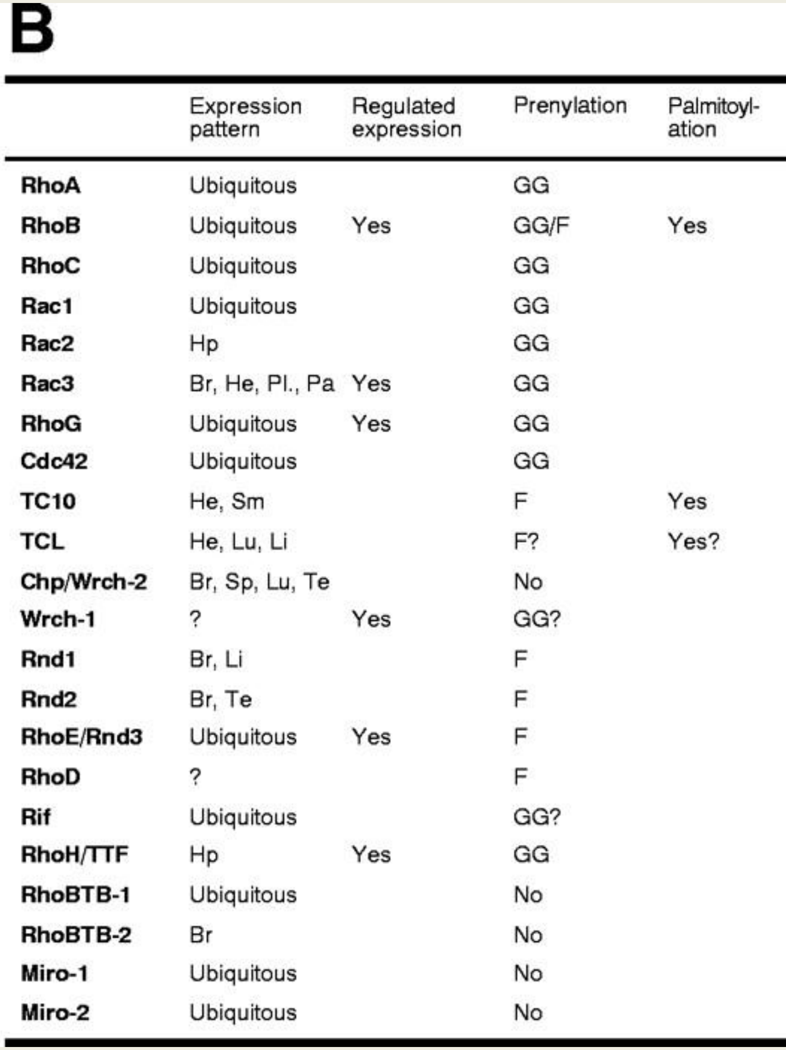
Four ways of maintaining differentiation after the initial signal has been given (Part 4)



DEVELOPMENTAL BIOLOGY, Eighth Edition, **Figure 6.38 (Part 4)** © 2006 Sinauer Associates, Inc.

Post-translational modification and expression patterns of Rho proteins.




B

	Expression pattern	Regulated expression	Prenylation	Palmitoylation
RhoA	Ubiquitous		GG	
RhoB	Ubiquitous	Yes	GG/F	Yes
RhoC	Ubiquitous		GG	
Rac1	Ubiquitous		GG	
Rac2	Hp		GG	
Rac3	Br, He, Pl., Pa	Yes	GG	
RhoG	Ubiquitous	Yes	GG	
Cdc42	Ubiquitous		GG	
TC10	He, Sm		F	Yes
TCL	He, Lu, Li		F?	Yes?
Chp/Wrch-2	Br, Sp, Lu, Te		No	
Wrch-1	?	Yes	GG?	
Rnd1	Br, Li		F	
Rnd2	Br, Te		F	
RhoE/Rnd3	Ubiquitous	Yes	F	
RhoD	?		F	
Rif	Ubiquitous		GG?	
RhoH/TTF	Hp	Yes	GG	
RhoBTB-1	Ubiquitous		No	
RhoBTB-2	Br		No	
Miro-1	Ubiquitous		No	
Miro-2	Ubiquitous		No	

Wennerberg K , Der C J J Cell Sci 2004;117:1301-1312

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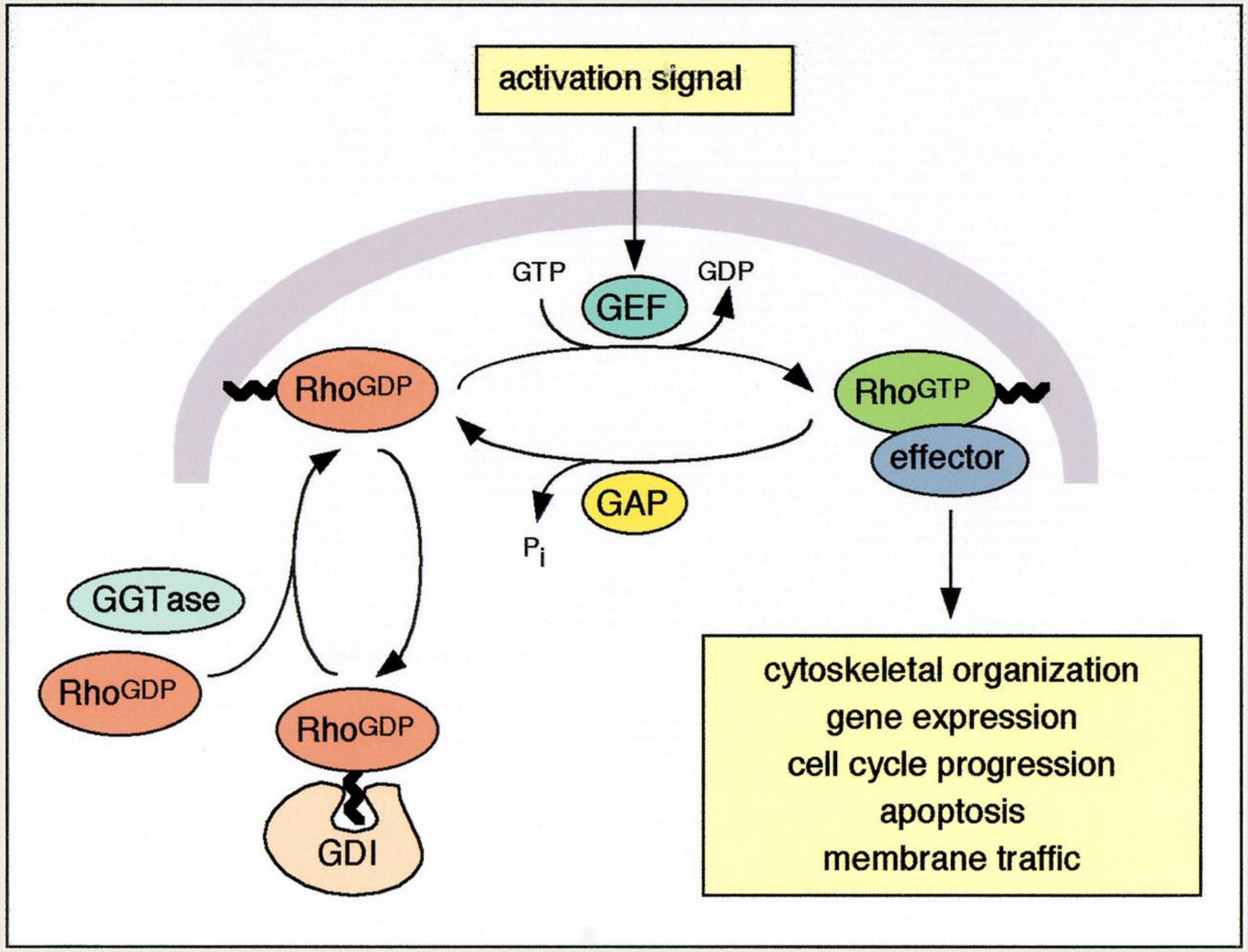
The Rho GTPase switch.



cytoskeletal organization
gene expression
cell cycle progression
apoptosis
membrane traffic

Schmidt A , Hall A Genes Dev. 2002;16:1587-1609

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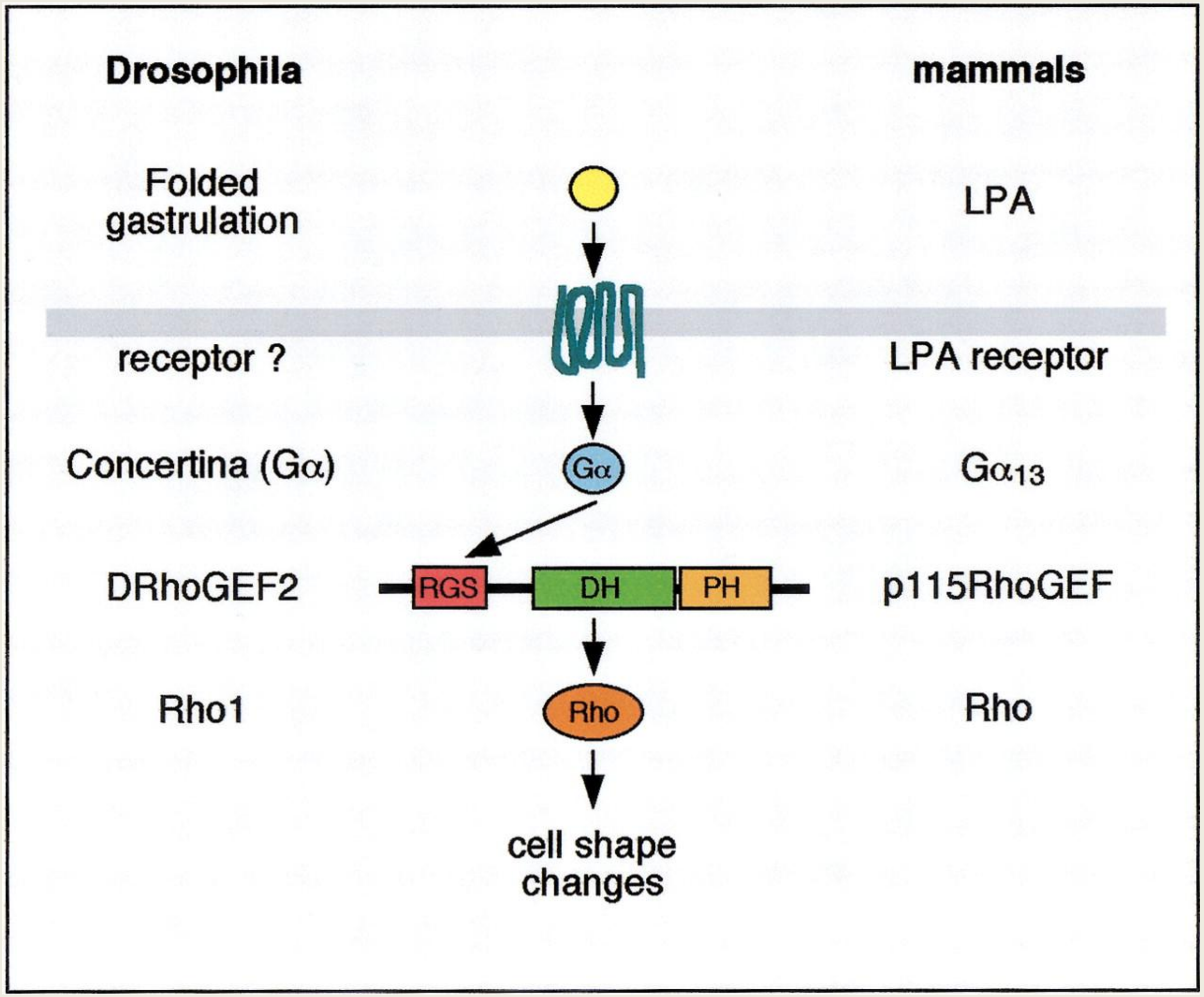
Conserved signaling pathways in Drosophila and mammalian cells control Rho-dependent cell shape changes.



Schmidt A , Hall A Genes Dev. 2002;16:1587-1609

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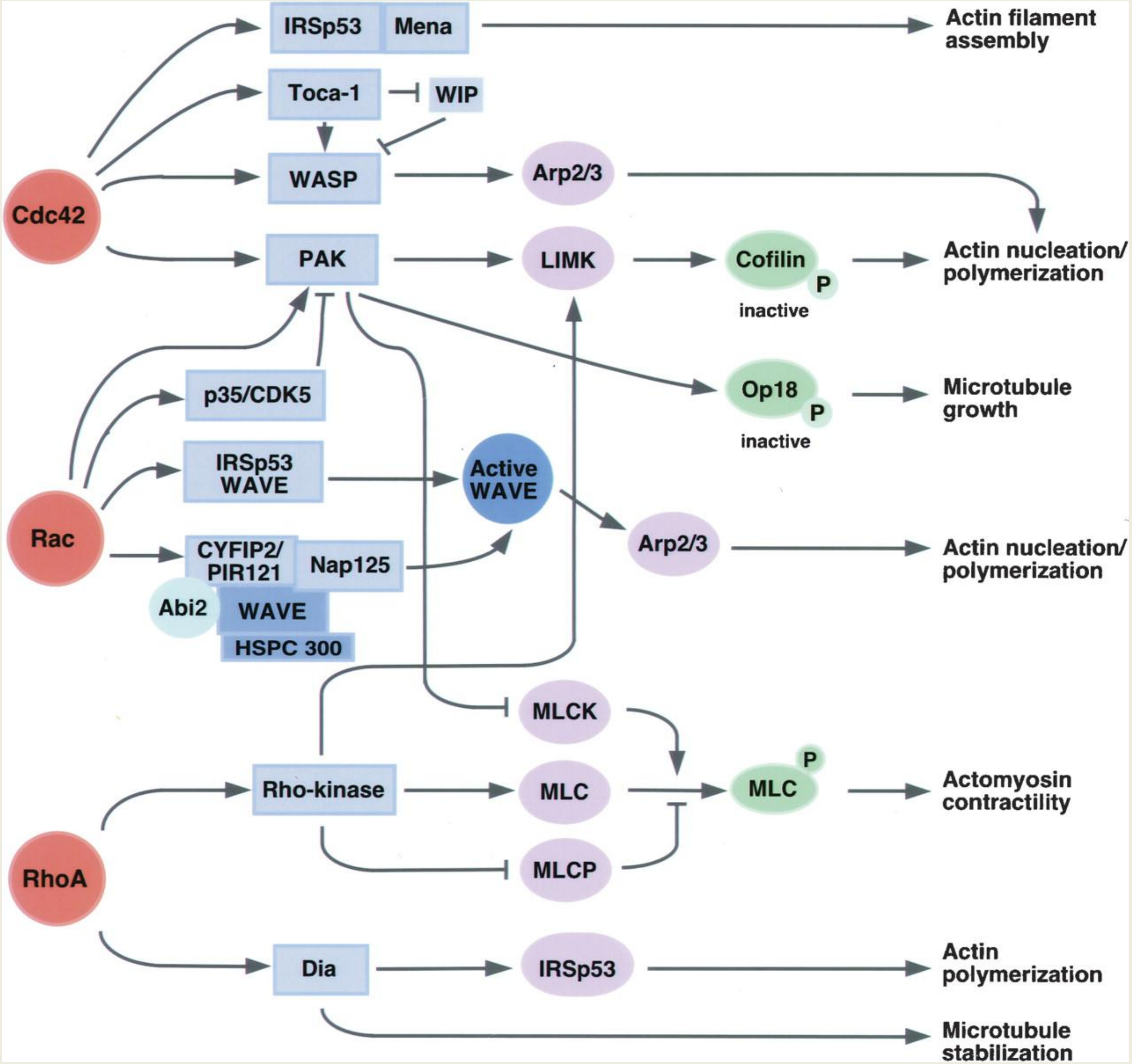
Rho GTPase effectors implicated in actin and microtubule dynamics.



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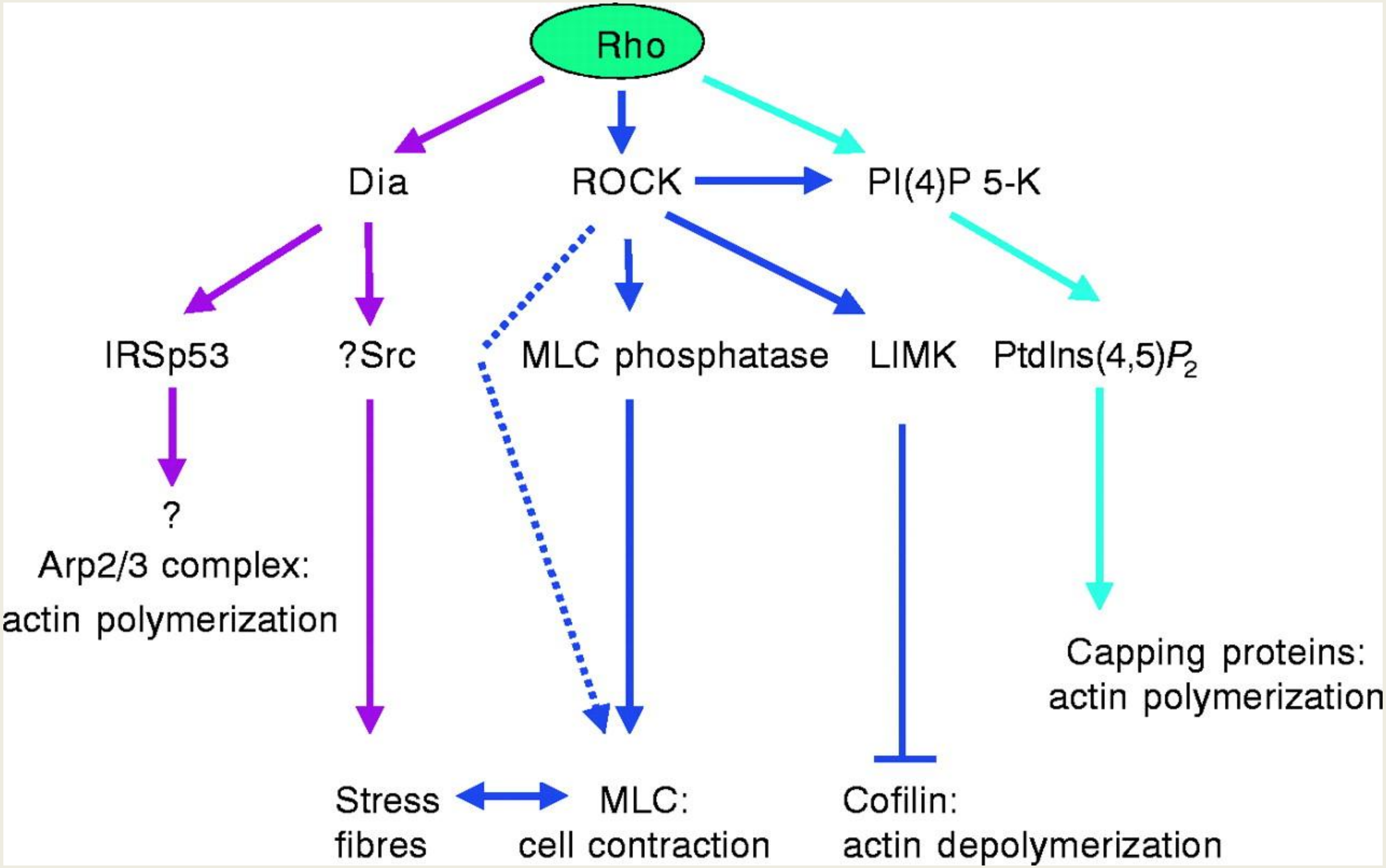
Govek E et al. Genes Dev. 2005;19:1-49

Some targets for Rho linked to actin reorganization.



Ridley A J J Cell Sci 2001;114:2713-2722

Journal of Cell Science
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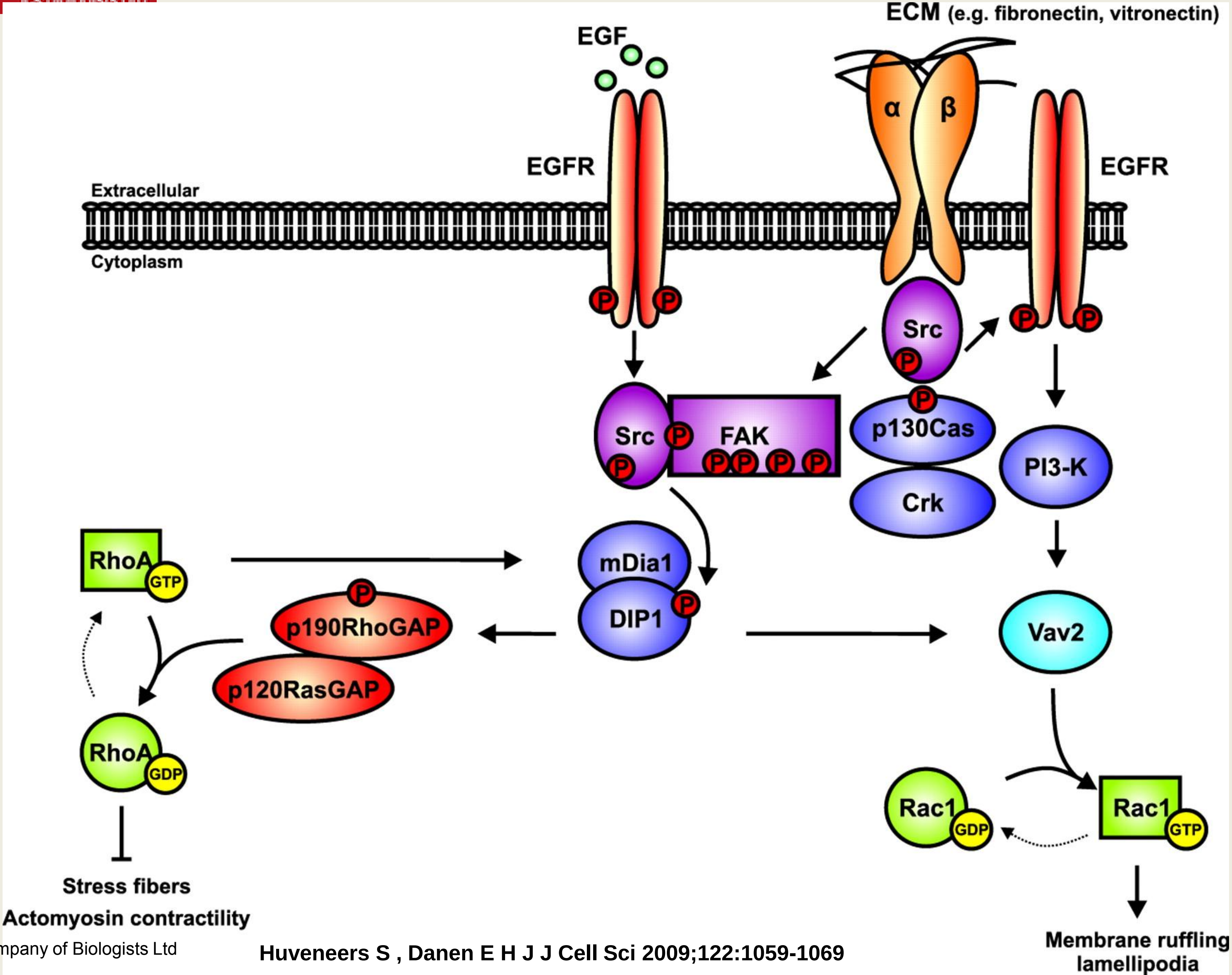
Journal of Cell Science

Crosstalk between integrins and EGFR regulates Src activation to control Rho-GTPases.



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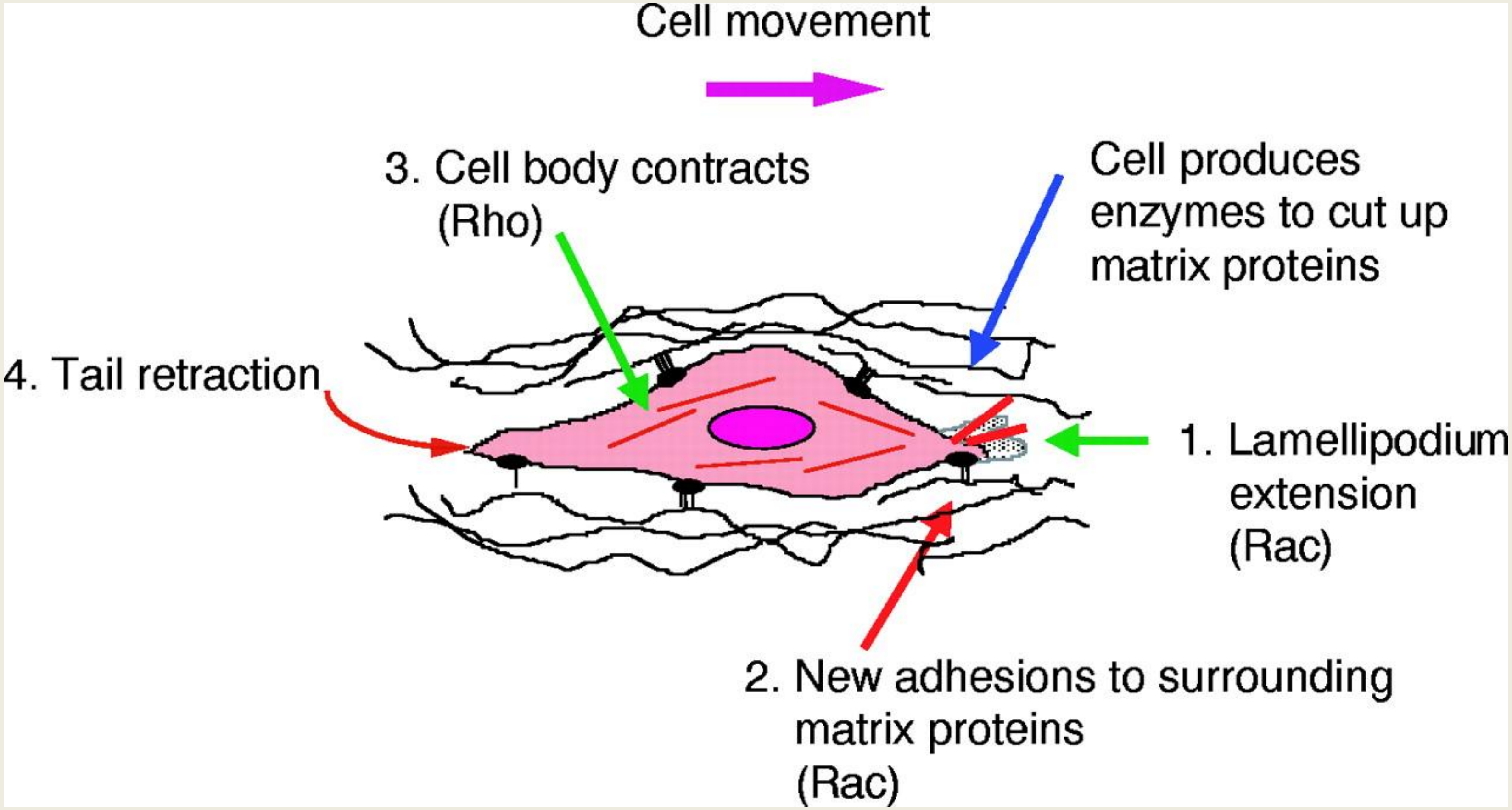
Huveneers S , Danen E H J J Cell Sci 2009;122:1059-1069

A model for the steps of cell migration.



Cell movement
3. Cell body contracts (Rho)
Cell produces enzymes to cut up matrix proteins
4. Tail retraction
1. Lamellipodium extension (Rac)
2. New adhesions to surrounding matrix proteins (Rac)

Ridley A J J Cell Sci 2001;114:2713-2722

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Overview of some signal transduction pathways:

→ 1. **MAP kinases:**

→ 2. **PI3K/Akt:**

} Cell growth, death, development, and differentiation.

3. **JAK / STAT**: Cell growth / immune response / differentiation.

4. **Wnt:** Cell fate during development and oncogenesis.

5. **Others:** Non cell membrane receptors (eg. steroids), receptor serine/threonine kinases and ion-channels.

Resource: www.cellsignal.com/references/pathways

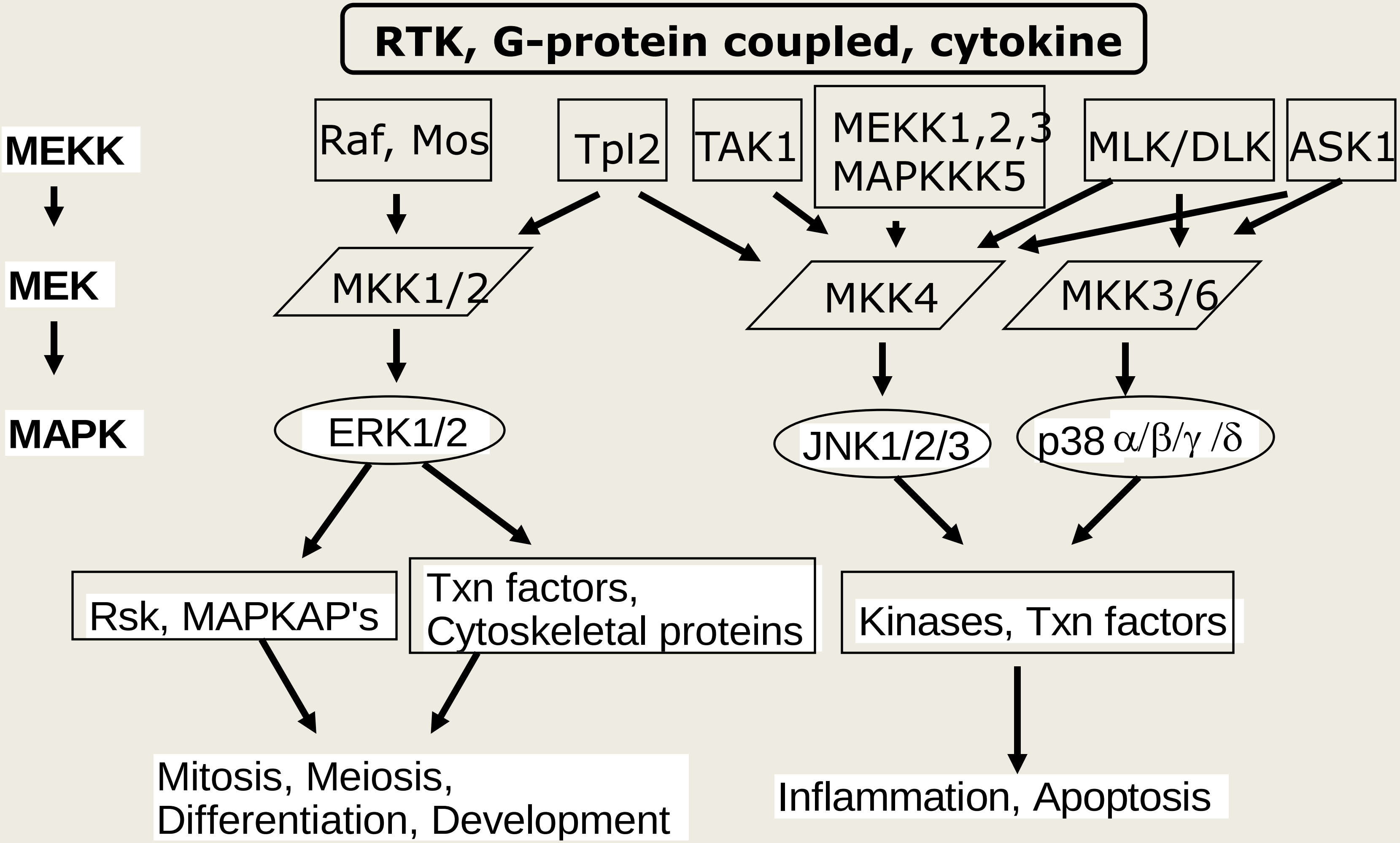
MAP Kinase Signaling Pathways

Growth / Differentiation

Growth factors, neurotransmitters, vasoactive peptides, cytokines

Stress Response

Inflammmatory cytokines, DNA damage, stress sensors



RTK, G-protein coupled, cytokine

MEKK → **MEK** → **MAPK**

MEKK: Raf, Mos; Tpl2; TAK1; MEKK1,2,3 MAPKKK5; MLK/DLK; ASK1

MEK: MKK1/2; MKK4; MKK3/6

MAPK: ERK1/2; JNK1/2/3; p38 $\alpha/\beta/\gamma/\delta$

Rsk, MAPKAP's

Txn factors, Cytoskeletal proteins

Kinases, Txn factors

Mitosis, Meiosis, Differentiation, Development

Inflammation, Apoptosis

miRNAs in vertebrate development: There are a lot unknown because the the lack of efficient methods to uncover the targets of miRNAs.

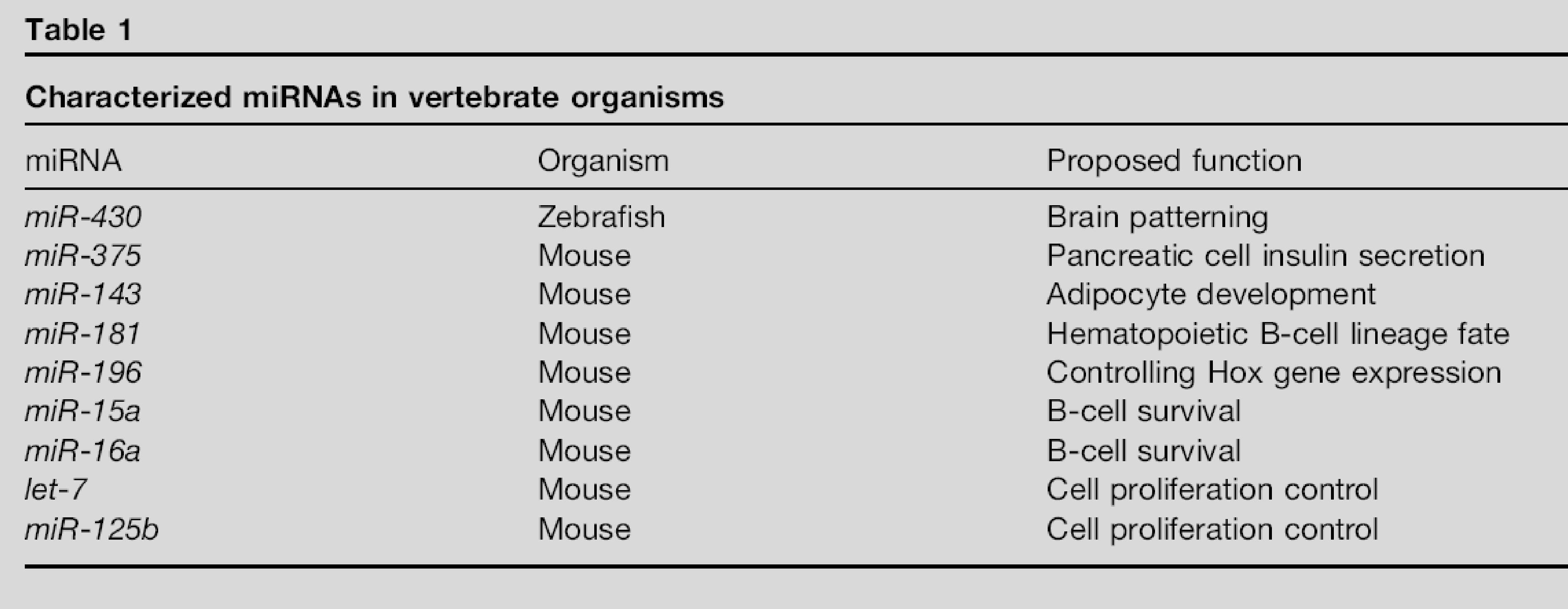


Table 1

Characterized miRNAs in vertebrate organisms

miRNA	Organism	Proposed function
miR-430	Zebrafish	Brain patterning
miR-375	Mouse	Pancreatic cell insulin secretion
miR-143	Mouse	Adipocyte development
miR-181	Mouse	Hematopoietic B-cell lineage fate
miR-196	Mouse	Controlling Hox gene expression
miR-15a	Mouse	B-cell survival
miR-16a	Mouse	B-cell survival
let-7	Mouse	Cell proliferation control
miR-125b	Mouse	Cell proliferation control

Current Opinion in Genetics & Development 2005, **15**:410–415

Expression of lin-4 allows *C. elegans* to proceed to the late developmental stage

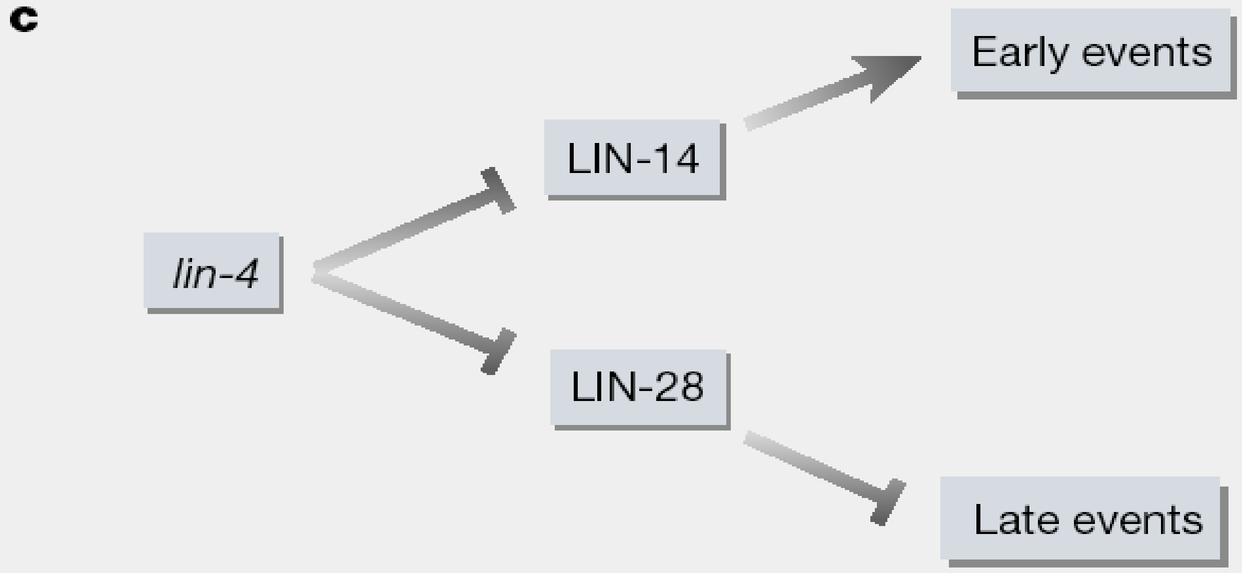
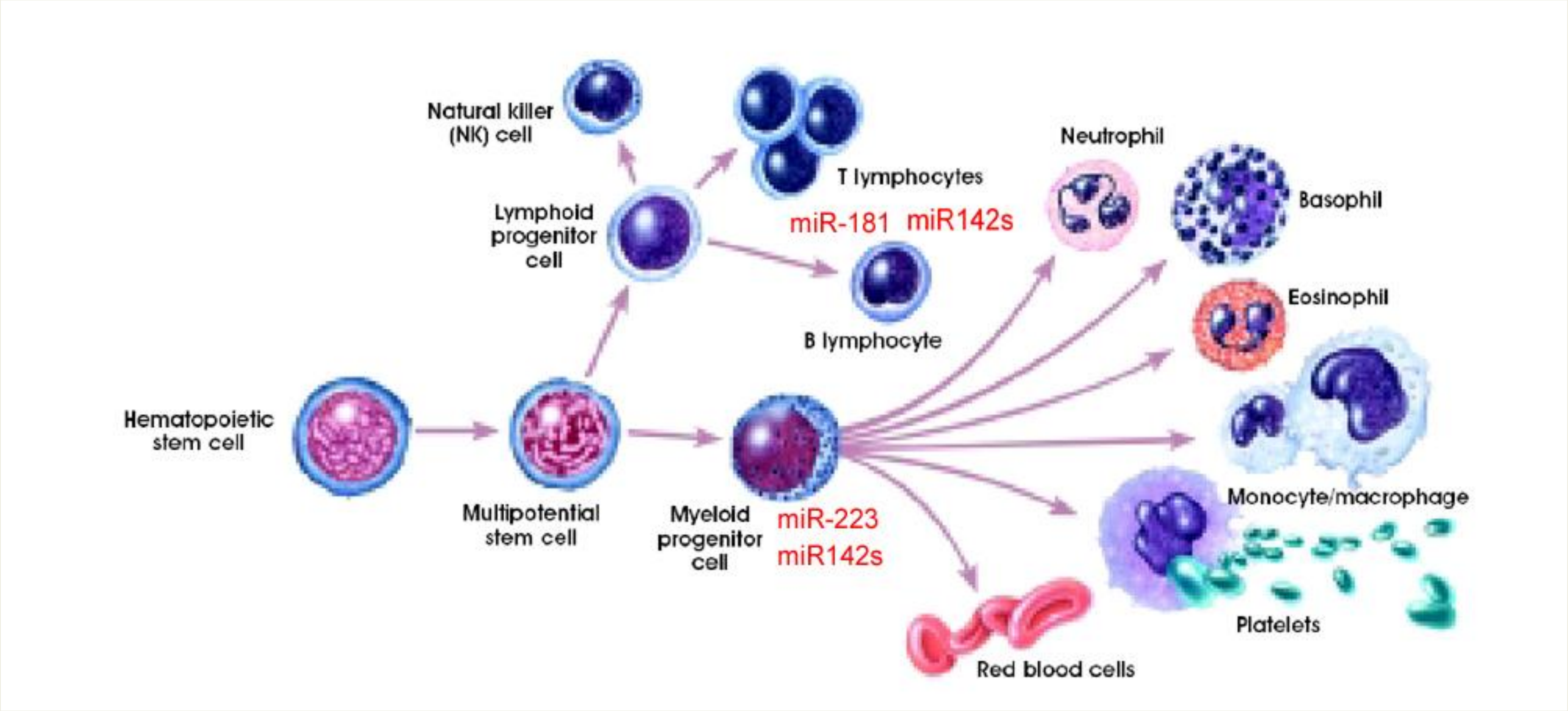


Figure 1 Examples of the imprecise base pairing of animal miRNAs with their targets. The *lin-4* miRNA is shown with its complementary sites in *lin-14* (**a**) and *lin-28* (**b**). There are several further complementary sites of imprecise base pairing in the 3′ UTR of *lin-14* (ref. 22; data not shown); only one site is predicted for *lin-4* in the *lin-28* 3′ UTR[24]. **c**, During larval development of *C. elegans*, *lin-4* coordinates the downregulation of LIN-14 and LIN-28 protein concentrations, which in turn regulates the expression of stage-specific developmental events.

miRNA controls the differentiation of the hematopoietic stem cell



(Science 2004)

Theories of cell aging

- DNA damage and DNA repair
  - Loss of repair effciency with age leads to somatic mutation with effects described above.
- Mitochondrial free radical theory
  - Damage to mitochondria and cellular proteins from free radicals generated in mitochondria causes cell aging.
- Altered proteins
  - Accumulation of damaged protein in cells causes cellular processes to work poorly.

Theories of Cell Aging

- **Wear and tear theory**: Little chemical insults and free radicals have cumulative effects
- **Immune system disorders**: Autoimmune responses and progressive weakening of the immune response
- **Genetic theory**: Cessation of mitosis and cell aging are programmed into genes. **Telomeres** (strings of nucleotides on the ends of chromosomes) may determine the number of times a cell can divide.

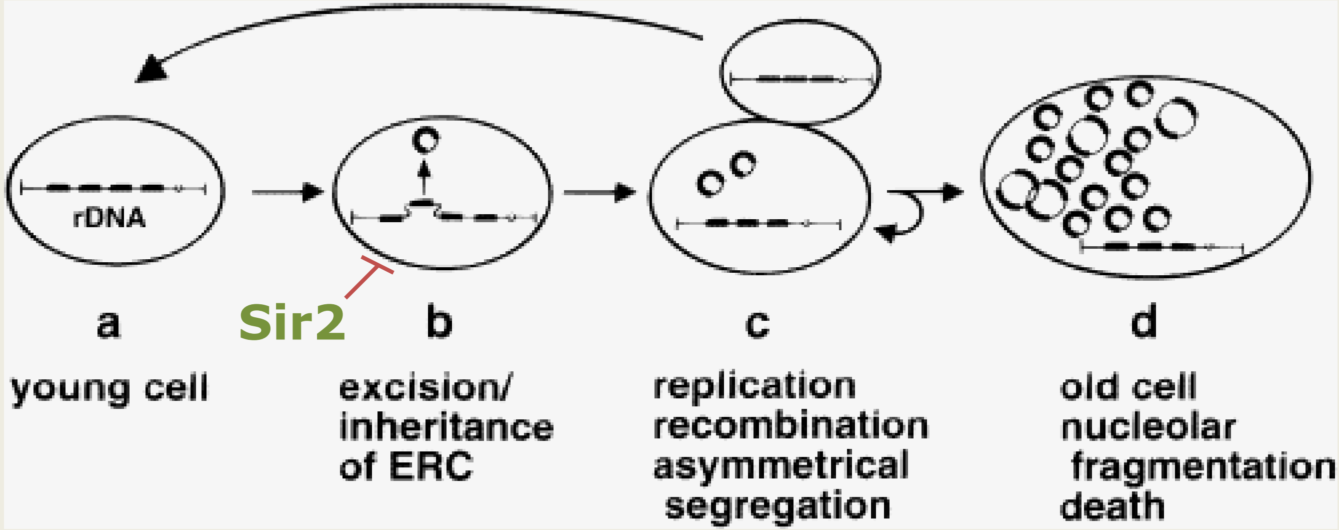
What is the yeast senescence factor?

1) extrachromosomal rDNA circles (ERCs)

2) dysfunctional mitochondria

3) oxidatively damaged proteins

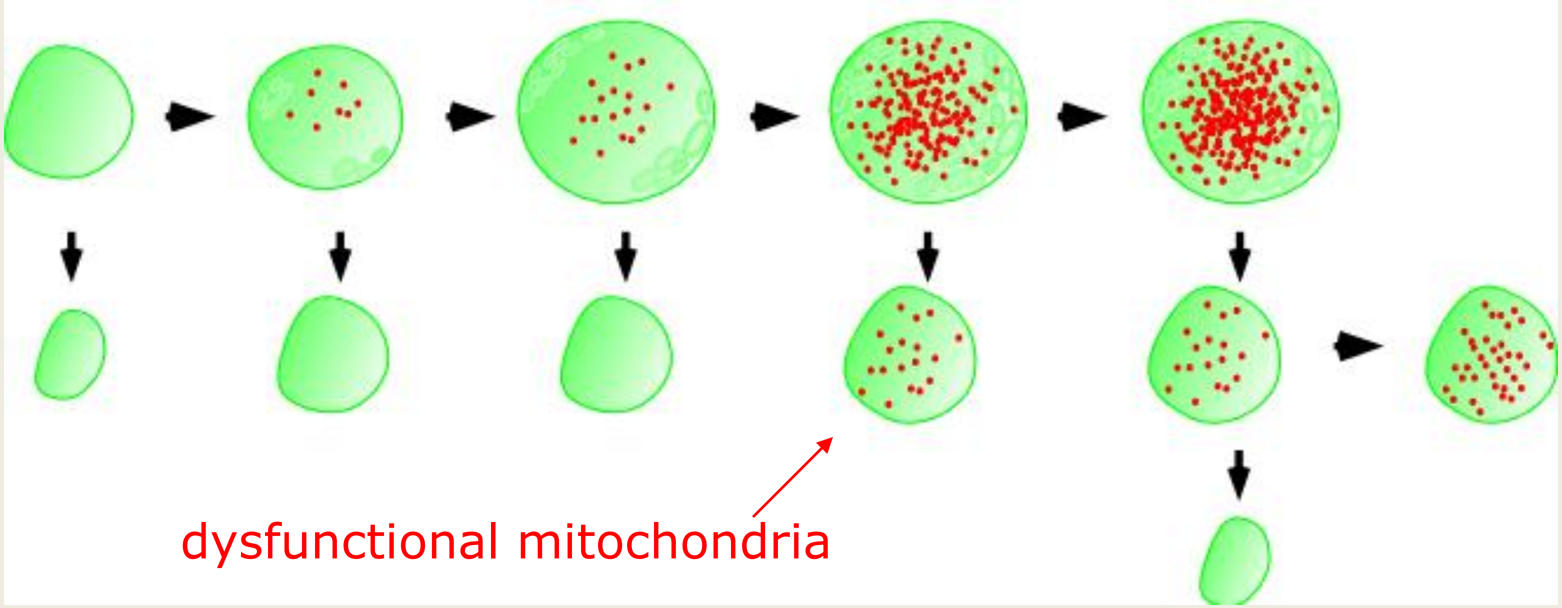
Extrachromosomal rDNA Circles as a cause of yeast aging



Sinclair and Guarente *Cell* 91:1033 (1997)

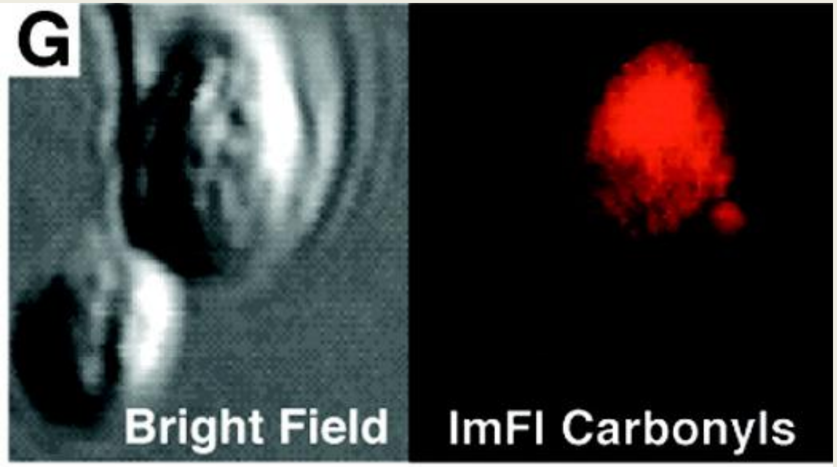
- Excised from the chromosomal array by recombination
- Recombination is suppressed by Sir2
- Replicate nearly every cell cycle
- Have a strong mother segregation bias at mitosis
- High levels can inhibit cell division
- Inherited by the daughters of old mothers

Dysfunctional mitochondria as a senescence factor



- Only mitochondria with newly-replicated mtDNA segregate to daughter cells; old ones stay in mother
- Old mother cells show signs of mitochondrial dysfunction and pass on dysfunctional mitochondria to their daughters

Is Damaged Protein a Senescence Factor?

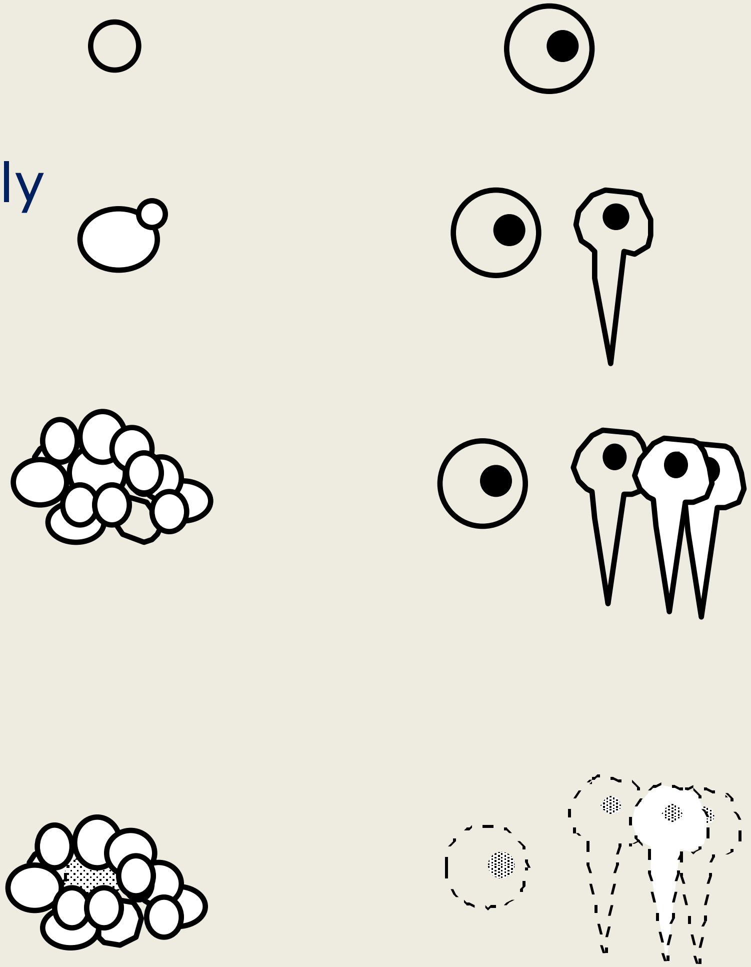


Aguilaniu, et al. *Science* 299:1751 (2003)

- Oxidatively damaged protein is preferentially segregated to mother cell
- Damaged protein accumulates in aging mother cells
- Past some threshold age (>10), it is inherited by the daughters of old mothers

How does Yeast Aging relate to Cellular Senescence in Humans?

- Telomere-independent
- Asymmetrically dividing cells
- For what cell type is this a model?



Stem cells:

- Express telomerase
- Divide asymmetrically
- Undergo senescence
- No ERCs!